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Assessment of Indiana bat reproductive condition, recapture trends, and temperatures of artificial roosts in Kentucky

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ASSESSMENT OF INDIANA BAT REPRODUCTIVE CONDITION, RECAPTURE TRENDS, AND TEMPERATURES OF ARTIFICIAL ROOSTS IN KENTUCKY

BY

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BY

MICHAELA L ROGERS

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ABSTRACT

Recovery of bat species impacted by white-nose syndrome (WNS) will necessarily require population growth. I assessed reproductive capability of the endangered Indiana bat (*Myotis sodalis*) in Kentucky, where WNS was first detected in hibernacula in April 2011. Due to loss of fat reserves associated with WNS infection, coupled with the energetic expenditures associated with pregnancy, I hypothesized mass of reproductively-active females captured during the maternity season would decrease across my data collection period (2017-2019). Further, I predicted that reproductive rates in the study population would be lower than historic rates for Kentucky. Mist net surveys around artificial maternity roosts at three focal management areas resulted in the capture of 866 Indiana bats across 22 netting events during the 2017-2019 maternity seasons. I examined changes in female mass (a measure of reproductive health) across years in order to determine how bats responded to WNS, and also assessed population trends in female pregnancy and lactation timing over the course of a season. A linear mixed model (LMM) was used to examine the effects of year (an analog for WNS impact) and wing score on the mass of female bats during the maternity season within my study term. To assess and account for the influence of other variables (reproductive condition, right-forearm length, capture date, site) on female mass, I conducted Akaike's Information Criterion (AIC) model selection and restricted analysis to relevant models. Generalized linear mixed models (GLMM) with binomial distribution were implemented to determine if reproductive proportion differed between my field-collected data versus historic records for Kentucky. Counter to expectations, data at the population-level indicate 92% of adult female Indiana bat captures exhibited signs of reproduction (pregnancy,

lactation, or post-lactation). Further, juvenile bats comprised 55% of captures in surveys during the post-parturition phase of the maternity season, and multiple juveniles were recaptured as reproductive adults during the study. Although reproductive proportion trended lower in post-WNS captures (92% compared to 99% pre-WNS) no significant difference was found between pre and post-WNS reproductive proportions. Across the three years of my study, post-WNS within-colony reproductive proportions did not differ. These data suggest reproductive potential has been sustained in the studied colonies despite bats' exposure to WNS during hibernation; promising evidence for persistence of the species. Even so, optimized management of maternity habitat remains critical to support population recoveries from WNS.

Additionally, I reported band recovery data of bats at the study colonies. While banding provides valuable information on migratory behavior, travel distances, survival rates and reproductive rates, among other behavioral insights, band recovery rates are persistently low. My study was designed to apply bands in large numbers to determine recapture rates at focal management locations of the Indiana bat during the maternity season, and to investigate bat movement within and from these locations. Relying on the same mist net surveys as my first chapter, a total of 119 recaptures were observed at an overall recapture rate of 14%. Within-year recapture rates in individual years of the study increased from 1% in 2017 to 18% in 2018, leveling at 17% in 2019. Most recaptures at each site occurred within June of each year. Recaptures at the more intensively sampled Veterans Memorial Wildlife Management Area (VWMA) demonstrated occurrences of roost switching and movement between three discrete clusters of roosts. Recoveries were recorded in 4 Kentucky hibernacula, two of which (Bat and Saltpeter Caves) ranked

within the 10 most populous Indiana bat hibernacula in the 2019 range-wide population survey. Links were also revealed between a hibernaculum in Indiana and two capture sites, Bernheim Arboretum and Research Forest (BARF) and Yellowbank Wildlife Management Area (YWMA). Five of these recoveries were recorded in Jug Hole Cave, reported as the second largest Indiana bat hibernaculum in 2019 range-wide surveys. Most female hibernacula recaptures with known reproductive condition (80%) showed signs of reproduction upon first capture. Additionally, two females exhibited reproductive capability prior to and following their recovery in caves. These observations demonstrate the ability of female Indiana bats to continue the migratory cycle despite WNS-related energy depletions coupled with expenditures of pregnancy, and provide evidence that female Indiana bats can engage in reproduction following potential WNS exposure during the hibernation period.

Finally, I investigated temperature conditions and use of artificial roosts by Indiana bats. Deployment of artificial roost structures is an increasingly common strategy in bat research and management, and understanding potential benefits and impacts of these structures on roosting bats is imperative to management of imperiled species. Although all roosts fluctuate in temperature, roosts that remain cool (<15°C) for extended periods may cause bouts of torpor, potentially impacting females during maternal seasons. Overheating roosts (>40°C) can cause heat stress, while extreme overheating events cause mortality. I hypothesized that temperatures would differ across three roost clusters based on differing levels of solar exposure at our focal study site, VWMA, a location with well-documented presence of Indiana bats in Kentucky and discrete sections of BrandenBark™ artificial roosts spread across the landscape. I also predicted

that live potential roost trees at the site would be cooler than artificial roosts, and when comparing artificial habitat only, that bats would be found in greater densities at warmer artificial roosts due to preference for solar exposed snags. To record roost temperatures, data loggers were placed on the exterior of false bark on roost structures at northeast and southwest aspects (n = 38) and set to capture hourly temperature through the maternity season. Data loggers were placed in the same orientation on one live tree in each cluster (n = 6). Bat use of structures was indexed via standardized guano screens, with guano collected every 2-3 days from different clusters. Generalized linear models (GLM) approximating a gamma distribution and AIC model selection processes were used to determine the most influential variables on daily mean, minimum, and maximum roost temperature. Mann-Whitney U tests were conducted to determine if artificial roosts differed in temperature from live trees. Graphing approaches were implemented to explore relationships between temperature and roost use by bats.

North, Central and South roost clusters did not vary significantly from one another in temperature profile, and aspect placement of roost data loggers did not cause deviations in mean, minimum, or maximum daily temperature of roosts. Comparisons between live trees and artificial roosts resulted in no significant difference between daily mean and minimum temperature, while daily maximum temperature was slightly higher at artificial roosts. No temperature measurements were collected that exceeded the maximum temperature threshold of 40°C. Daily minimum temperature for individual loggers fell below the minimum threshold on 648 instances across 29 days, representing temperatures across all roosts, leading to the conclusion that bats at VWMA are under

greater threat from roosts falling under 15°C than overheating. However, results may have differed had loggers been placed on the interior of false bark on roosts.

Bats exhibited the highest densities at the South cluster of roosts, followed by the Central and North clusters. However, the colony appeared to spread across VWMA throughout the season, and cumulative collection from the greatest-use roost in each cluster accounted for 36% of all guano collected in the study. The lack of significant temperature difference between clusters, and persistent use of all roost clusters at VWMA indicates each provides suitable habitat for Indiana bat maternity colonies. All artificial roosts were used at VWMA in 2019 and roost switching was confirmed, indicating suitability of roosts placed in a variety of habitat conditions, and the ability of bats to successfully relocate. Reproduction and pup rearing were observed at all three clusters, indicating the importance of variation in roost placement on the landscape for opportunistic selection. Selection of roosts by Indiana bats is likely the result of additional unknown factors.

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INTRODUCTION

The federally endangered Indiana bat's (*Myotis sodalis*) vulnerability to natural and anthropogenic disturbance is exacerbated by specific hibernacula and roost requirements (Humphrey 1978, Endangered Species Preservation Act 1966, United States Fish and Wildlife Service (USFWS) 2007). Improper forest management, snag reduction and disruption of limited winter hibernacula historically culminated in the federal listing of the species so that management actions would be designed to mitigate population declines (Lacki et al. 2007). More recently, the Indiana bat became subject to further declines across its range following the introduction of white-nose syndrome (WNS) to North America in 2006. The fungal agent responsible, *Pseudogymnoascus destructans* (*Pd*), presents itself on exposed membranes of the wings, muzzles and ears of infected bats (Blehert et al. 2009, Lorch et al. 2011). Mortality from WNS is attributed to disturbance of hibernating individuals. The inability to remain in torpor while afflicted by *Pd* leads to depleted energy sources, leaving heavily affected bats to endure the resource-limited season with little to no fat reserves (Blehert et al. 2009). *Pd* spreads rapidly, and the clustering behavior of bats in hibernation promotes high transmission rates across colony-forming species (Langwig et al. 2012). The fungus can persist in cave or mine environments for long periods of time, even in the absence of bats (Lorch et al. 2012, Hoyt et al. 2015). Monitoring of known hibernacula has confirmed active spread of WNS across at least a dozen cave-associated species,

including the Indiana bat, where it has caused severe population declines (Frick et al. 2010, White-nose Syndrome Response Team 2020). Significant reduction of individuals surviving hibernation will compromise species' ability to retain a stable population. Further, Indiana bat recovery will rely heavily on population growth to compensate for regional depletions (Thogmartin et al. 2013). Therefore, research on current reproductive trends is critical in ensuring practical recovery efforts.

The persistent survivorship of adult bats despite WNS exposure does not ensure reproductive capability in the spring (Pettit and O'Keefe 2017). Parturition in Indiana bats occurs between late June and early July, wherein females typically birth a single pup (Thomson 1982). To preserve energy during hibernation for spring reproduction, female bats are necessarily energy efficient in comparison to male bats (Jonasson and Willis 2011). Although this may confer a greater probability of survival during hibernation, afflicted females must initiate more severe spring recovery responses requiring additional energy expenditures (Meierhofer et al. 2018). For females, entering reproductive periods with depleted energy levels may result in the inability of gravid females to carry pups to term, or the forgoing of pregnancy entirely (Jonasson and Willis 2011, Francl et al. 2012, Pettit and O'Keefe 2017).

An inhibited ability to complete reproductive cycles due to WNS occurs in the Indiana bat, little brown bat (*Myotis lucifugus*), northern long-eared bat (*Myotis septentrionalis*) and big brown bat (*Eptesicus fuscus*) (Pettit and O'Keefe 2017). Further, capture rates of non-reproductive female Indiana bats within WNS-impacted populations have increased, and lower proportions of juveniles have been captured for several *Myotis* species (Francl et al. 2012, Pettit and O'Keefe 2017). Finally, shorter

durations are exhibited within each reproductive period (pregnancy, lactation, post-lactation), and shifts in earlier timing of pregnancy and lactation occur among bats captured in areas where WNS has already spread (Francl et al. 2012). Although some studies have observed reproductive viability for WNS-inflicted *Myotis* species, the various lines of evidence presented in these studies underscore uncertainty regarding the severity of compromised reproductive potential (Meierhofer et al. 2018, O'Keefe et al. 2019). Across the Indiana bat's range, undisturbed hibernacula and availability of maternity roosts are essential for the species to successfully reproduce and persist through winter. Kentucky contains the highest number of hibernacula for Indiana bats in North America, several of which represent critical habitat within the distribution of the species. For example, Bat Cave and Saltpeter Cave are hibernacula in eastern Kentucky, and collectively contained 7% of the overall population estimate for the Indiana Bat in the winter of 2019 (USFWS 2019). Indiana bats banded in Illinois, Indiana, Michigan, Ohio, Pennsylvania, and Tennessee during the summer have been reported overwintering in Kentucky hibernacula, demonstrating established links across the species' range (Zack Couch, Kentucky Department of Fish and Wildlife Resources (KDFWR), pers. communication). Due to the state's importance for both the overwintering and summer breeding ranges for the species, it is critical to consider the reproductive health of colonies in Kentucky.

My objective was to investigate reproductive trends of WNS-impacted Indiana bat colonies in Kentucky, where WNS was first detected in April 2011 (USFWS 2011). Kentucky has lost over 15,000 Indiana bats since 2007, while range-wide, the Indiana bat population has declined by 19.2% between 2007 and 2019 counts (USFWS 2019). I

assessed the health and reproductive status of focal maternity colonies to identify potential reproductive changes in the wake of WNS. I hypothesized: i) mass of reproductively-active females captured during the maternity season would decrease across my data collection period (2017-2019) due to the assumed widespread presence of WNS across Kentucky hibernacula after introduction to cave systems in the state (White-Nose Syndrome Response Team 2019), and in comparison to pre-WNS conditions (based on pre-existing data); ii) the proportion of reproductively active female bats within populations will have declined coincident with the spread of WNS in Kentucky; and iii) for those bats able to engage in reproduction, WNS exposure would result in phenological shifts (earlier and shorter periods) in when typical maternal transitions would occur.

STUDY AREA

Study sites were selected based on the presence of historic maternal use by Indiana bats, a determining factor in the installation of BrandenBark™ artificial roosts (Copperhead Consulting, Paint Lick, KY) prior to the onset of this study. Artificial roosts (Fig. 1)[1] used by bats consisted of a 7.6 m untreated utility pole with a layer of polyurethane false bark surrounding the tops of poles (Gumbert et al. 2013). In total, three sites were targeted across Kentucky (Fig. 2).

Bernheim Arboretum and Research Forest

Bernheim Arboretum and Research Forest (BARF) is located in Bullitt County, KY (Fig. 2), within the Knobs-Norman Upland Region (Level IV Ecoregion). Regional

[1] All figures and tables are presented in the appendices of this document.

elevation ranges from 115-457 m, and topography consists of rounded hills, knobs and ridges containing mixed deciduous forest. High gradient valleys are common and generally narrow, with a few swamp-laden valleys between knobs separating the Bluegrass Region (Woods et al. 2002). Forested areas consist of oak-hickory (*Quercus-Carya*) with mesic uplands containing beech (*Fagus grandifolia*), white oak (*Quercus alba*), tulip-poplar (*Liriodendron tulipifera*), sugar maple (*Acer saccharum*), and northern red oak (*Quercus rubra*) (Woods et al. 2002). Surrounding agriculture includes cropland and pasture. The ownership covers 5,868 ha of park containing native forest, grassland, glade habitat, streams and other water bodies (Berry 2016, Bernheim Arboretum and Research Forest 2019). The roost area at BARF is adjacent to a restored creek and consists of four artificial roosts ca. 20 m from one another.

Veterans Memorial Wildlife Management Area

Veterans Memorial Wildlife Management Area (VWMA) spans 1,011 ha in the Inner Bluegrass Region (Level IV Ecoregion). Extensive karst is present in the region ranging from 152-304 m in elevation, characterized by almost level to rolling upland plain topography (Woods et al. 2002). The region's upland forests consist of open forest remnants of oaks and ash, areas of oak-hickory forest and drainages of oak-maple forests. Box elder (*Acer negundo*), tulip poplar (*Liriodendron tulipifera*), black locust (*Robina pseudoacacia*), hackberry (*Celtis occidentalis*) sweetgum (*Liquidambar styraciflua*), pin-oak (*Quercus palustris*) and mulberry (*Morus*) are common across the region (Woods et al. 2002). Surrounding land use activities include agriculture, suburban development, and horse and livestock pastures. Managed by KDFWR in Scott County, KY (Fig. 2), VWMA supports hardwood forest habitat as well as shrubland,

grassland, wetland and pond habitat (KDFWR 2016a). Artificial roosts at VWMA are spread across three distinct areas of the study site; each roost cluster was regarded as a discrete roost location. These are referred to as VWMA North, Central and South clusters. Roost clusters are located ca. 0.5-1.5 km from one another while roosts within each cluster are located ca. 30 m apart. In 2017 and 2018 there were 18 roosts at VWMA (n = 6 roosts per cluster). In March 2019, all roosts except one were replaced due to degradation of the wooden utility poles (North n = 6, Central n = 7, South n = 6 roosts per cluster), totaling 19 roosts at VWMA.

Yellowbank Wildlife Management Area

Yellowbank Wildlife Management Area (YWMA) is located on 2,736 ha in Breckinridge County, KY (Fig. 2) within the Crawford-Mammoth Cave Uplands (Level IV Ecoregion). Elevation in the region ranges from 105-290 m and is characterized by hills, cliffs and broad karst valleys (Woods et al. 2002). The region's upland forest is oak-hickory dominated with white oak, black oak (*Quercus velutina*), post oak (*Quercus stellata*) and pignut hickory (*Carya glabra*), while mesic sites contain forests with beech, sugar maple, white ash (*Fraxinus americana*) southern red oak (*Quercus falcata*), and tulip poplar (Woods et al. 2002). Cropland and livestock pastures are common in surrounding areas (Woods et al. 2002). YWMA is managed by KDFWR and consists primarily of upland deciduous forests, farmland and bottomland forest habitat (KDFWR 2016b). Artificial roosts are located within the Town Creek Tract of YWMA. This study site contains ten artificial roosts arranged in 2 clusters (<200 m from one another) of 5 roosts ca. 15 m from one another.

METHODS

Bat Capture

Mist net surveys were conducted at all study sites across the maternity seasons from 2017-2019. Surveys spanned a 15-day study period (June 1-15) annually; additional surveys took place at YWMA and VWMA in mid-July of 2017, and efforts increased to include BARF the following two years in order to check for the presence of juveniles in study populations (July 8-26).

On a given survey night, a single artificial roost was targeted, with mist nets arranged on three sides surrounding the roost (Fig. 3). Specifically, nets were deployed using a 'three high' rope and pulley system (allowing nets to a height of ca. 8.5 m), with one side of the roost having 6 m nets and the other sides of the roost having 4 m nets (i.e., the nets generally flanked the roost in the shape of an isosceles triangle). A stake was driven into the ground ca. 2 m adjacent to one of the sides of the triangle so that a support pole could be placed onto this stake and the net triangle could be opened if deemed necessary (and then lowered once all bats were removed), reducing further captures from bats exiting the roost.

Capture procedures followed Indiana bat summer survey guidelines specified by USFWS (2017), and were authorized by EKU IACUC 05-2018. All surveys were authorized and reported under KDFWR's federal Endangered Species Act section 6 permit. Age and sex characteristics of bats were recorded based on established procedures of illuminating wings to examine bones for closure of the epiphysial plates, and by examining individuals externally for male or female reproductive organs (Brunet-Rossini and Wilkinson 2009, Silvy 2012). Reproductive status (pregnant,

lactating, post-lactating, testes descended, non-reproductive) and morphometrics (right forearm length (RFA), total mass) were measured as outlined in Haarsma (2008) using digital calipers (Traceable Products, Webster, TX) and digital platform scales (Pesola, Schindellegi, Switzerland). Wing score from 0 (no visible wing damage) to 5 (very poor with large tears) was determined as per Reichard and Kunz (2009), and a UV light was used to fluoresce potential white-nose lesions present on exposed membranes of captured bats (Turner et al. 2014). Individuals were banded before release with a uniquely numbered 2.9 mm lipped bat ring provided by KDFWR (Porzana Ltd., East Sussex, UK).

Data Analysis

To determine whether WNS influenced adult female mass, an analog for WNS impact on bat health (McGuire et al. 2018), I created linear mixed models (LMM) using body condition and WNS factors. Square root transformation was performed on response variable data (mass) to approximate normality and fit was confirmed ($\chi^2 = 1$, df = 540). Prior to evaluation, I constructed 7 models based on ecologically-relevant hypotheses attempting to account for variables (year of capture, RFA length, reproductive condition, wing score, Julian date, site) impacting mass of female bats (Table 1). Variables were partitioned into either body morphology categories (reproductive condition, RFA), which depict factors expected to impact the mass of bats with or without the presence of WNS, and WNS factors (wing score, year). Bats assigned higher wing scores (>0) were expected to be of lower mass due to the damage of WNS, while continued exposure across years was expected to lead to decreased bat mass. Site and Julian day were modeled as random factors to account for the possibility

that site-specific differences may affect female mass and condition, and the common influence of date on the reproductive cycle of all bats within the study. Body morphology and WNS factors were paired in models to account for the impacts of both on bats, while also creating WNS only models to elucidate the impact of these focal factors on female bat mass. I used Akaike's Information Criterion (AIC) to guide model selection (using R packages lme4 and AICmodavg, R version 3.6.2, R Core Team 2019) and restricted inferences to relevant models ($\Delta AIC_c < 2$) (Burnum and Anderson 2002). Model averaging in R package MuMIN was used to determine competitive model covariates, and predictors were considered significant if confidence intervals did not cross 0 ($\alpha = 0.15$). Confidence intervals were calculated at 85% as defined in Arnold (2010) to be most compatible with AIC model selection, ensuring inclusion of informative parameters. Complex model development and ranking was restricted to post-WNS data, as inclusion of pre-WNS records would have required reliance on incomplete and minimal data.

To determine if the proportion of reproductively viable bats decreased in post-WNS captures, pre-WNS capture data were retrieved from the KDFWR bat database (Traci Hemberger, KDFWR, pers. communication). This database compiles data from state issued scientific collection permit submissions, research records, monitoring reports, and museum records from throughout the state, and includes records dating to 1874. Pre-WNS records used in my analyses were collected from 1979-2010 between June 4th and July 17th (n = 79). Records of adult female Indiana bats caught within June and July with reproductive condition listed were chosen, while records without sex or age identified were culled from the dataset. A generalized linear mixed model (GLMM)

with a binomial distribution was used to compare broad reproductive proportion data between the pre- and post-WNS datasets (R package lme4). Data were grouped by county, which was modeled as a random factor. WNS was the predictor variable in this model, in which the data were identified as either the pre- or post-WNS category based on a cutoff year of 2010, the year before WNS was confirmed in Kentucky (White-nose Syndrome Response Team 2019). The response variable was the reproductive proportion out of total captures. A GLMM was also used to discern any difference between individual years of post-WNS captures. Post-WNS data were grouped by site, modeled as a random factor with year as the sole predictor variable. The response variable was the reproductive proportion out of total captures. An ANOVA (anova function in base R) was used to determine statistical significance of the aforementioned GLMMs, as each was compared against the null model. All means are presented as ± SE and all statistical tests were conducted in RStudio (RStudio Team 2016, R Studio version 1.1.419).

To address whether timing of reproductive events has been shortened or if transitions from each stage are occurring earlier following the spread of WNS, the first observation, last observation, and duration of each portion of the reproductive cycle was charted for all available pre-WNS and post-WNS records. The proportion of captures within each reproductive class were then compared graphically in June and July across both capture periods.

RESULTS

In total, 22 mist net surveys were conducted across the three years of study. Specifically, 5 surveys were conducted at BARF, 11 surveys at VWMA, and 6 surveys

at YWMA (Table 2). BARF surveys consisted of a single June survey per year from 2017 to 2019, with one survey in July during 2018 and 2019. Surveys were conducted at two VWMA sites (North and South) in June of 2017, which increased to surveys at all three sites in 2018 and 2019, with VWMA North surveyed in July of all three years to confirm juvenile presence. YWMA was surveyed in June and July for each of the three years of the study. An average of 39 ± 3 Indiana bats were caught per netting night. Regarding non-target captures, only 1 evening bat (*Nycticeius humeralis*) and 3 big brown bats (*Eptesicus fuscus*) were captured alongside 866 Indiana bats (both species at BARF on 6/8/2017, and an individual big brown bat at YWMA on 6/4/2018, and at BARF on 6/10/2019. Of the total Indiana bat captured, 698 were adults and 168 were juveniles.

Adult female Indiana bats exhibited signs of reproduction at a rate of 92% (pregnant, lactating, or postlactating; Fig. 4; $n = 547$), and juveniles comprised 55% ($n = 168$) of captures in surveys during the post-parturition phase of the maternity season over the entire survey period (Fig. 5). Adult females recaptured across survey years ($n = 61$) were reproductively active at both capture instances at a rate of 89%, while under 10% were non-reproductive at first capture and then reproductively active upon recapture, and fewer than 2% of female recaptures were non-reproductive at both captures (Fig. 6). All female juveniles recaptured as adults ($n = 8$) exhibited evidence of reproduction (Fig. 7).

WNS Impact on Bat Mass

Adult female bat mass, by reproductive condition, across the post-WNS survey period averaged 8.91 ± 0.03g for pregnant, 6.98 ± 0.07g for lactating, 6.72 ± 0.11g for

post-lactating, and 6.93 ± 0.10g for non-reproductive bats. Across all reproductive classes, adult female bat mass across the post-WNS survey period averaged 8.27 ± 0.05g (Fig. 8). AIC selection procedures to examine factors influencing mass did not result in a single model with unambiguous support. Rather, three models ranked with a $\Delta AIC_c < 2$, accounting for a cumulative weight of 0.86 (Table 1) (Table 3). Model averaging provided evidence that RFA, reproductive condition, wing score and year were influential variables (Table 4). Pregnant reproductive condition (in comparison to other reproductive statuses) and larger RFA each associated positively with female bat mass (Fig. 9). Within the year covariate class, 2019 showed a negative association with female bat mass only when compared to captures in 2018 (Table 4). Wing scores of 1 were negatively associated with female bat mass; this wing score was observed across all three years of study, and in each reproductive class (Figs. 10-12).

Reproductive Rate Pre- and Post-WNS

Pre-WNS capture records indicate 99% of adult female Indiana bats were reproductive, while post-WNS records indicate a reproductive proportion of 92% (Fig. 13). The global model used for analysis of pre-WNS vs post-WNS reproductive proportions fit a binomial distribution ($\chi^2 = 1$, df = 15). The global model was not significantly different from the null model ($\chi^2 = 0.16$, df = 1, P = 0.69), indicating no difference between pre-WNS and post-WNS reproductive proportions. In individual years in post-WNS surveys the reproductive proportion was similar from 2017 to 2018 (89% to 90%, respectively) and increased to 96% in 2019. The global model used for post-WNS reproductive proportion analysis fit a binomial distribution ($\chi^2 = 0.99$, df = 5). This model, used to determine if the reproductive proportion differed between the

three years, showed no significant differences from the null model ($\chi^2 = 0.28$, df = 2, P = 0.87), indicating minimal difference in reproductive proportion from 2017 to 2019.

Phenological Shifts in Reproduction

Within the June and July capture months, pre-WNS and post-WNS reproductive stage observations all followed expected phenology (Thomson 1982) for pregnancy, lactation and post-lactation periods (Fig. 14). Lactating bats were observed across an 18-day longer timespan in the post-WNS dataset as compared to pre-WNS captures. Post-lactating bats were observed across the same duration (16 days), though post-lactating bats in the post-WNS dataset were observed 9 days later than the pre-WNS dataset. Pregnant bats were first captured within three days of one another in both datasets; the span of pregnant captures appeared shorter in the post-WNS dataset, a consequence of the June 15th cutoff date used for the surveys in my study.

Pre-WNS maternal captures in June consisted of 60% lactating bats and 40% pregnant bats, in comparison to rates of 13% lactating and 87% pregnant in post-WNS bats (Fig. 15). July maternal captures for pre-WNS and post-WNS bats were consistent, with 68% and 65% of maternal captures identified as lactating, and 32% and 35% identified as post-lactating, respectively.

DISCUSSION

Based on three years of field sampling, and counter to expectations, the mass of reproductively active females captured during the maternity season did not decrease across the post-WNS study term. Regardless of WNS impact, the observed relationship between RFA and reproductive condition with adult female mass followed biometric expectations. Further, the observed relationship between reproductive condition and

mass similarly followed morphological expectations. Although the year model received strong support, the year effect was weakly negative from 2018 to 2019. This effect was unsupported by the average weight of adult female bats within each of these two years (8.36 ± 0.07g, 8.34 ± 0.07g, respectively). The consistency of adult female masses across my survey years suggests the ability of females to gain weight across consecutive maternity seasons has not been compromised following presumed WNS exposure during hibernation. The ability of afflicted bats to retain fat stores and conserve energy is a key adaptation for persistence in the face of WNS (Jonasson and Willis 2011, Cheng et al. 2018). Female bats in these study colonies seemingly are persisting despite additional energy constraints from WNS. Further, a subset of individuals exhibited reproductive capacity across several maternity seasons. These are promising results. For continuous reproductive success, the summer period is likely critical for mass recovery before re-exposure to *Pd* in the fall.

WNS-impacted bats were observed in each of the studied colonies (i.e., wing scores > 0). Although this parameter associated negatively with female bat mass, wing scores of 1 were spread across reproductive classes and throughout the three years of study. Notably, equal proportions (8%) of bats assigned either a 0 or 1 wing score were non-reproductive. This refutes my expectation that non-reproductive bats, if unable to engage in reproduction based on energetic constraints, may exhibit more frequent instances of WNS impact (Pettit and O'Keefe 2017). However, due to the high percentage of pregnant and lactating bats captured, most wing scores of 1 were also found in these categories.

My data suggest the proportion of reproductively active female bats within populations has not significantly declined coincident with the spread of WNS in Kentucky. However, the initial two years of my post-WNS monitoring, 2017 and 2018, demonstrated declines of 8-9% when compared individually with the pre-WNS proportion. The higher proportions of reproductively viable females observed in 2019 in comparison to the prior two years of my mist net surveys may suggest initial population-level recovery from WNS. While potential variation in year to year samples cannot be ruled out, these data align with a previous study of bats surviving WNS. As individual bats able to withstand impacts of *Pd* are selected, WNS-impacted individuals are expected to produce more viable offspring (Langwig et al. 2017). Initial instances of resistance have been reported in little brown bat colonies (*Myotis lucifugus*), in which bats persisting post-WNS were more resistant to the growth of *Pd*, increasing individual chances of survival into the next reproductive season (Langwig et al. 2017). Additionally, preliminary evidence indicates genetic changes in little brown bat populations known to have survived WNS infection which, while preliminary, may suggest adaptation-driven selection (Auteri and Knowles 2020). Although the fungal load of my three focal colonies is unknown, there is clear evidence of survival despite likely WNS exposure due to the widespread nature of WNS, coupled with known interchanges of sites with WNS positive or presumed positive (based on proximity to other positive sites) hibernacula in the three Indiana bat colonies (see Chapter 2). Females recaptured in later survey years provide evidence of survival after WNS exposure during hibernation, in addition to reproductive capability. Recaptured Indiana bats (n = 61) exhibited a high rate (89%) of consistent reproductive instances across

multiple years, with an additional 9.8% recaptured as reproductive during one capture. Initial captures of bats as juveniles provided evidence of recruitment and the ability of offspring produced by adults in these colonies to reach reproductive viability. Although the full, persistent impacts of WNS on bat populations are, as yet, unknown, the ability of Indiana bats to have survived and reproduced at a rate of 92% in this study is indicative of reproductive persistence within these colonies.

Counter to expectations for reproductive bats, WNS exposure did not result in marked phenological shifts across reproductive phases. Although differences were observed regarding the length of time lactating bats were captured during the post-WNS period, lactating periods shorten instead of lengthen for bats exposed to WNS (Francl et al. 2012). If patterns followed expectations of earlier and shorter reproductive phases, post-lactating bats post-WNS would have been captured starting earlier than the pre-WNS population due to the shorter lactation window. Reproductive proportions in my study were also contrary to expectations according to WNS impacts. It would be expected that energy-depleted post-WNS populations would contain the higher percentage of lactating individuals in June (as opposed to pre-WNS), due to the inability to carry a longer term pregnancy pre-WNS (Francl et al. 2012). However, the absence of data from late June in the post-WNS dataset likely influenced these proportions. I concede the absence of data from May for the post-WNS period is a limiting factor of this study, as additional conclusions may have been drawn from extended reproductive period data.

The findings of this study are promising; however, limitations in the dataset must be acknowledged. Pre-WNS analyses were restricted to records available in

KDFWR databases, and adult female Indiana bats consisted of a small sample size (n = 79) in comparison to the post-WNS dataset (n = 547). Further, pre-WNS records were from a period when fewer surveys were conducted at either natural or artificial roosts; nevertheless, I assume data collected through mist netting would provide an accurate representation of overall reproductive condition for the species. Data from the pre-WNS period was greater in the latter years of the collection period, from 2005-2010, which may not have permitted detection of longer term changes relative to earlier surveys. Additionally, monitoring of juveniles in the post-WNS study period was limited to a singular capture event at a given site in July, which does not ascertain survival through the entirety of the season. While my study provides measures of reproductive capability, this may or may not reflect actual reproductive success. Due to the widespread nature of WNS across eastern North America, this study acts on assumption of likely exposure to some level of WNS in the environment. Additionally, this study was conducted during the summer period, wherein many individuals clear visible signs of WNS infection due to elevated body temperatures above the range of *Pd* growth (Verant et al. 2012, Langwig et al. 2014). Though a maternal study could not be conducted during the winter when exposure to WNS occurs in cave environments, studies during this time period may elucidate more information on individual-level WNS damage.

MANAGEMENT IMPLICATIONS

Understanding the full biological response a disease invokes in a species is imperative in managing for recovery (Hoyt et al. 2016). WNS impacts in important Indiana bat hibernacula in Kentucky are well-documented elsewhere through winter surveillance (USFWS 2019). My study provides a maternal season-specific measure of

post-WNS reproductive capacity in a linchpin location within the species' distribution. Despite evidence of WNS impact on individual bats, reproductive capability was sustained in my study colonies. Obviously, the recruitment of juveniles into recovering populations will play an essential role in future recovery. Netting efforts at Indiana bat roosts should continue in order to provide additional information on how Indiana bat populations continue to adapt to WNS. Management of maternity habitat resources should remain in critical focus to support the recovery of inflicted bat populations.

CHAPTER 2: RECAPTURE TRENDS IN MATERNITY COLONIES OF INDIANA BATS IN KENTUCKY

INTRODUCTION

The application of bands to the legs and wings of animals is a common practice when studying wildlife (Silvy 2012). Specifically, banding is a common identification method in mark-recapture studies of bats, and bands are widely applied in surveys as markers of previous capture (Ellison 2008). Since the first recorded instances of bat banding in 1916, and increased expansion of a program in North America in the 1930s, banding has provided information on homing behavior, return rates, travel distances, longevity, seasonal migrations, hibernation ecology, mortality and survival rates, and reproductive behavior of bats (Ellison 2008). However, despite widespread efforts, recapture success of banded bats is continually low. For example, during the term of the Bat Banding Program (BBP), a widespread banding effort including the United States, Canada, Mexico and Central America, 59,000 recoveries were reported out of 1,119,114 banded individuals, a rate of 5%, though the program ran from 1932 to 1972 (Ellison 2008). While individual records provide useful insight, small sample sizes limit inferences regarding bat ecology and management.

Several reasons likely underlie low bat recapture rates. Survey efforts, and coincidental band recoveries, are often linked with radio-tracking studies, or when surveys are concentrated in caves or other enclosed localities (Gumbert et al. 2002). When radio-tracking, the short time window (i.e., several weeks) following original capture does not provide information on demographic changes or site fidelity on annual

bases, unless bats happens to be recaptured and tracked the following year in multi-year studies. Further, funding opportunities for long-term studies that increase recovery chances are sparse. Additionally, bands may fall off, or become damaged and illegible (Ellison 2008). Finally, bats are migratory and thus can travel hundreds of kilometers from original banding sites (Roby et al. 2019). Despite these challenges, banding is used by bat biologists and facilitated by state resource agencies across North America. For example, over 18,000 bats have been banded in Kentucky from 1996 - present (Traci Hemberger, KDFWR, pers. communication).

Despite low recapture rates, existing recapture records yield valuable information about how bats utilize summer and winter habitats. For example, banding has documented the return of female bats to summer maternity roosts over consecutive years (Kurta and Murray 2002), and has allowed assessment of hibernaculum fidelity and travel distances during fall migration (Davis and Hitchcock 1965). Kentucky contains the most Indiana bat hibernacula sites per state, and has experienced the fourth greatest net loss of the species since 2007 (USFWS 2019). Moreover, two of the state's hibernacula, Bat Cave and Saltpeter Cave in Carter County, ranked within the top ten most populous Indiana bat hibernacula in the 2019 range-wide population survey (USFWS 2019). The proximity of critical overwintering habitat for the endangered species indicates the importance of analyzing recovery records to determine links between maternity sites and hibernacula.

Banding has also been used to examine summer roost switching and recurrent site use when large netting efforts are conducted, though this is most frequently done by pairing banding efforts alongside radio-telemetry efforts. Further, banding has allowed

estimation of the longevity of bats, providing evidence that bats may live longer than 20 years (Greenhall and Paradiso 1968). The longest-lived Indiana bat (*Myotis sodalis*), a focal species for research and management in the eastern United States, was captured 20 years after it was first banded (LaVal and LaVal 1980). While more directed research is needed to solidify our knowledge of survival and age structure of bats (USFWS 2007), the demographic data yielded by recapture records are indicative of the varied uses afforded by recapture records for bats.

Considering the need for more thorough understanding of the benefits of bat banding, I designed a study that allowed for the application of a large number of bands on concentrated maternity colonies of federally endangered Indiana bats at multiple sites in Kentucky. These sites have a history of maternal use by Indiana bats, and the highly philopatric nature of the species was expected to allow for greater recaptures upon yearly consecutive capture events at the sites (Humphrey et al. 1977). My objectives were: i) determine recapture rates at focal management locations of the Indiana bat during the maternity season, and ii) investigate bat movement within-site, and iii) report on movement and distances traveled from these locations to hibernacula.

STUDY AREA

Study sites were selected based on the presence of historic maternal use by Indiana bats, which was coincident with the presence of BrandenBark™ artificial roosts (Copperhead Consulting, Paint Lick, KY). Artificial roosts (Fig. 1) used by bats consisted of a 7.6 m untreated utility pole with a layer of polyurethane false bark surrounding the tops of the poles (Gumbert et al. 2013). In total, three sites were targeted across Kentucky (Fig. 2).

Bernheim Arboretum and Research Forest

Bernheim Arboretum and Research Forest (BARF) is located in Bullitt County, within the Knobs-Norman Upland Region (Level IV Ecoregion). Regional elevation ranges from 115-457 m, and topography consists of rounded hills, knobs and ridges containing mixed deciduous forest. High gradient valleys are common and generally narrow, with a few swamp-laden valleys between knobs separating the Bluegrass Region (Woods et al. 2002). Forested areas consist of oak-hickory (*Quercus-Carya*) with mesic uplands containing beech (*Fagus grandifolia*), white oak (*Quercus alba*), tulip-poplar (*Liriodendron tulipifera*), sugar maple (*Acer saccharum*), and northern red oak (*Quercus rubra*) (Woods et al. 2002). Surrounding agriculture includes cropland and pasture. The ownership covers 6,530 ha of park containing native forest, grassland, glade habitat, streams and other water bodies (Berry 2016, Bernheim Arboretum and Research Forest 2019). The roost area at BARF is adjacent to a restored creek and consists of 4 artificial roosts ca. 20 m from one another.

Veterans Memorial Wildlife Management Area

Veterans Memorial Wildlife Management Area (VWMA) spans 1,011 ha in the Inner Bluegrass Region (Level IV Ecoregion). Extensive karst is present in the region ranging from 152-304 m in elevation, characterized by almost level to rolling upland plain topography (Woods et al. 2002). The region's upland forests consist of open forest remnants of oaks and ash, areas of oak-hickory forest and drainages of oak-maple forests. Box elder (*Acer negundo*), yellow poplar, black locust (*Robina pseudoacacia*), hackberry (*Celtis occidentalis*) sweetgum (*Liquidambar styraciflua*), pin-oak (*Quercus palustris*) and mulberry (*Morus*) are common across the region (Woods et al. 2002).

Surrounding land use activities include agriculture, suburban development, and horse and livestock pastures. Managed by KDFWR in Scott County, VWMA supports hardwood forest habitat as well as shrubland, grassland, wetland and pond habitat (KDFWR 2016). Artificial roosts at VWMA are spread across three distinct areas of the study site, and each roost cluster was regarded as a discrete roost location. These are referred to as VWMA North, Central and South clusters. Roost clusters are located ca. 0.5-1.5 km from one another while roosts within each are located ca. 30 m apart. In 2017 and 2018 there were 18 roosts at VWMA (n = 6 roosts per cluster). In March 2019, all roosts except one were replaced due to degradation of the wooden utility poles, totaling 19 roosts at VWMA (North n = 6, Central n = 7, South n = 6 roosts per cluster).

Yellowbank Wildlife Management Area

Yellowbank Wildlife Management Area (YWMA) is located on 2,736 ha in Breckinridge County, within the Crawford-Mammoth Cave Uplands (Level IV Ecoregion). Elevation in the region ranges from 105-290 m and is characterized by hills, cliffs and broad karst valleys (Woods et al. 2002). The region's upland forest is oak-hickory dominated with white oak, black oak (*Quercus velutina*), post oak (*Quercus stellata*) and pignut hickory (*Carya glabra*), while mesic sites contain forests with beech, sugar maple, white ash (*Fraxinus americana*) southern red oak (*Quercus falcata*), and tulip poplar (Woods et al. 2002). Cropland and livestock pastures are common in surrounding areas (Woods et al. 2002). YWMA is managed by KDFWR and consists primarily of upland deciduous forests, farmland and bottomland forest habitat (KDFWR 2016). Artificial roosts are located within the Town Creek Tract of

YWMA within an open canopy wetland adjacent to Town Creek. This study site contains 10 artificial roosts arranged in 2 clusters (<200 m from one another) of 5 roosts ca. 15 m from one another.

METHODS

Mist net surveys were conducted at all study sites across the maternity seasons from 2017-2019. Surveys spanned a 15-day study period (June 1-15) annually; additional surveys took place at YWMA and VWMA in mid-July 2017 to ascertain the presence of juveniles in the population, and efforts increased to include BARF the following two years (July 8-26).

On each survey night, a single artificial roost was targeted, with mist nets arranged on three sides surrounding the roost (Fig. 3). Specifically, nets were deployed using a 'three high' rope and pulley system (allowing nets to a height of ca. 8.5 m), with one side of the roost having 6 m nets and the other sides of the roost having 4 m nets (i.e., the nets generally flanked the roost in the shape of an isosceles triangle). A stake was driven into the ground ca. 2 m adjacent to one of the sides of the triangle so that a support pole could be placed onto this stake and the net triangle could be opened if deemed necessary (and then lowered once all bats were removed), reducing further captures from bats exiting the roost. Capture procedures followed Indiana bat summer survey guidelines specified by USFWS (2017), and were authorized by EKU IACUC 05-2018. All surveys were authorized and reported under KDFWR's federal Endangered Species Act section 6 permit. Age and sex characteristics of bats were recorded based on established procedures of illuminating wings to examine bones for closure of the epiphysial plates, and by examining individuals externally for male or

female reproductive organs (Brunet-Rossini and Wilkinson 2009, Silvy 2012). Reproductive status (pregnant, lactating, post-lactating, testes descended, non-reproductive) and morphometrics (right forearm length (RFA), total mass) were measured as outlined in Haarsma (2008) using digital calipers (Traceable Products, Webster, TX) and digital platform scales (Pesola, Schindellegi, Switzerland). Wing score from 0 (no visible wing damage) to 5 (very poor with large tears) was determined as per Reichard and Kunz (2009), and a UV light was used to fluoresce potential white-nose lesions present on exposed membranes of captured bats (Turner et al. 2014). Individuals were banded before release with a uniquely numbered 2.9 mm lipped bat ring provided by KDFWR (Porzana Ltd., East Sussex, UK).

To address my first objective, I compared recapture rates graphically across sites and years to determine if rates increased steadily each year (as I expected to yield a higher recapture rate with additional sampling effort). My second objective was addressed by examining trends in roost switching rates between the three distinct roost locations at VWMA, and investigating the effect of distance on bat movement between sites and overwintering grounds. Additionally, observations that link sites to specific hibernacula were reported.

RESULTS & DISCUSSION

In total, 22 mist net surveys were conducted across the three years of study. An average of 39 ± 3 (± SE) Indiana bats were caught per netting site (Table 5). In total, 1 evening bat (*Nycticeius humeralis*) and 3 big brown bats (*Eptesicus fuscus*) were captured alongside 866 Indiana bats. A total of 119 recaptures were observed, resulting in a recapture rate of 14% (Table 6).

Study Area Recoveries

Counter to expectation, I did not observe a steady increase in recapture rates across survey years (Fig. 16). The total number of recaptured bats increased by 17% from 2017 to 2018. The percentage of recaptures then remained consistent, decreasing by 1% in 2019. Beyond delineating overall recapture trends, I investigated recaptures across years and within sites. Within-year recapture trends at BARF were similar between 2018 and 2019 (Fig. 17); most recaptures each year occurred in June, while fewer recaptures were observed in July. However, the decrease was more pronounced in 2018 than 2019 (42% versus 10%, respectively). I judge this was due to the smaller sample size in 2018 compared to 2019. Due to substantial captures of juveniles during the July netting period, I expected recapture rates to follow this decreasing pattern within a survey year.

The robust sampling effort at VWMA allowed for detailed comparison of early, mid, and late-June capture periods. The greatest recapture rate within each year occurred in mid-June (Fig. 18), increasing dramatically from early June in 2018 and to a far lesser extent in 2019 (46% versus 4%, respectively). July recaptures remained consistent between 2018 and 2019, with only a 1% decrease at VWMA, again likely due to the rate of juvenile captures during the post-parturition July netting period.

Finally, YWMA followed a similar pattern to BARF and VWMA in 2018. The recapture rate of YWMA decreased 12% from June to July (Fig. 19). In 2019, all of YWMA's within-year recaptures were caught in June, while no recapture records were collected in July. Like the other sites, I attribute this observation to the rate of juvenile

captures in June, but this also serves as evidence of colony movement throughout the cluster of roosts at YMWA.

Roost Switching at VWMA

The greater sampling effort at VWMA allowed for analyses of bat movement throughout the site's North, Central and South clusters. The greatest amount of capture overlap occurred between Central and South clusters (9%), followed by capture overlap between the North and Central clusters (8%) (Table 7). This followed expectations, as the Central cluster serves as a transitional area at the site and is closest in proximity to the South cluster (<0.5 km). Site overlap is relative, however, as all similarity percentages are low and sampling was more intensive at the North cluster. Thus, I judge there may be even more overlap at the Central site than observed. In two instances, individual bats were recaptured at all three VWMA roost sites. This example of roost switching exemplifies the need for multiple roost options at a given maternity roost locality, and a spread of roost alternatives across the surrounding landscape (Callahan et al. 1997).

Hibernacula Recoveries

Banding efforts at my 3 sites resulted in 7 recoveries as of March 2020 in Kentucky hibernacula (Fig. 20). Specifically, a post-lactating female originally banded at YWMA in July 2017 was later recovered in February 2018 ca. 27 km southeast at B&O Cave in Breckinridge County, KY. Later that year, this female was captured for a third time at YWMA and identified as lactating. This provided evidence of a female bat hibernating in a WNS-positive cave able to maintain reproductive capability across consecutive maternity seasons. Other recoveries at B&O Cave followed a similar

migratory pattern. A lactating female bat banded at YWMA in July 2017 was recovered at this hibernaculum in February 2020. Non-reproductive female and male bats banded at YWMA in June 2018 were also recovered during a February 2020 survey at B&O Cave.

Several other Kentucky hibernacula were represented within recapture records. A non-reproductive male bat initially banded in June 2017 at BARF was later recovered ca. 95 km away in January 2019 at Jesse James Cave in Edmonson County, KY. Jesse James Cave is principally recognized as a gray bat (*Myotis grisescens*) hibernaculum. A pregnant female bat banded at VWMA in June 2017 was recovered ca. 125 km east in January 2019 at Saltpeter Cave in Carter County, KY. Another pregnant female banded at VWMA in June 2018 was recovered ca. 124 km east the following January 2019 at Bat Cave in Carter County, KY. Bat and Saltpeter caves were both confirmed WNS positive in 2013 (Traci Hemberger, KDFWR, pers. communication).

Several bats banded within my surveys (n = 9) were recovered in Indiana hibernacula (Fig. 21). The earliest of these cave recoveries, a male bat captured in May 2016 at BARF by Copperhead Consulting (Paint Lick, KY), was recovered ca. 66 km northwest in 2017 in Jug Hole Cave (Harrison County, IN). I recaptured this male upon its return to BARF in June 2019. A second male bat, the only cave recovery initially banded as a juvenile, was captured in July 2017 at YWMA and was recovered ca. 42 km northeast in Jug Hole Cave in February 2019. Three female bats initially banded in 2018 at YWMA followed this pattern (one of which was banded in June, the other two in July) and were recovered at Jug Hole Cave in February 2019. One female was pregnant upon initial banding, another was non-reproductive, and the reproductive

status of the third female is unknown. The pregnant female was then recaptured the following June in 2019 at YWMA, again demonstrating consecutive years of reproductive capability. Jug Hole Cave was reported as the second largest Indiana bat hibernaculum in 2019 surveillance counts (USFWS 2019), and is the largest hibernaculum for the species in the state of Indiana (Brack and Brack 2019). These recaptures link two of my study colonies to a major Indiana bat overwintering site with likely WNS exposure (though not officially confirmed), due to its proximity to other WNS positive caves (Brack and Brack 2019).

Additional out of state recoveries include a pregnant female bat initially banded in June 2017 at BARF, recovered ca. 42 km northwest in February 2019 at Wallier Cave, a hibernaculum in Harrison County, IN. Several more individuals were recovered in Wyandotte Cave, a hibernaculum in Crawford County, IN. One of these, a male bat, was captured at YWMA in July 2017, and was recovered ca. 46 km northeast at the hibernaculum in February 2019. Two other recoveries, both captured as pregnant females at YWMA in June 2018, were recovered during this same February survey. Recoveries of bats at both Wallier Cave and Wyandotte Caves confirm links to out of state WNS positive caves (Brack and Brack 2019). Wyandotte Cave was the fourth largest Indiana bat hibernacula in 2019, hosting 10% of the total overwintering Indiana bat population (USFWS 2019).

Indiana bats migrate varying distances (Roby et al. 2019), and recovery records following my banding efforts underscore this variation. Most female hibernacula recaptures with known reproductive condition (80%, n =10) showed signs of reproduction at their original banding, demonstrating female ability to continue the

migratory cycle despite WNS-related energy depletions coupled with expenditures of pregnancy. Additionally, two females exhibited reproductive capability during the season prior to and following their recovery in caves. A single juvenile male hibernacula recovery highlights a known instance of a juvenile born within these colonies surviving several years post-capture and migrating to overwintering grounds. These data are promising, and the results from this study provide multiple lines of evidence that female Indiana bats can reproduce following potential WNS exposure during the hibernation period. Though encouraging, much of our reporting focuses on specific observations; future studies should continue to address gaps in knowledge regarding recapture success of bats. Importantly, data-sharing across state agencies can ensure recaptures are reported. Understanding links between summer maternity sites and hibernacula will aid in more comprehensive management of the Indiana bat, while highlighting population-level areas of overlap for the species.

CHAPTER 3: TEMPERATURE CONDITIONS OF ARTIFICIAL ROOSTS USED BY INDIANA BATS IN KENTUCKY

INTRODUCTION

The Indiana bat (*Myotis sodalis*) is specific in its maternal habitat requirements, preferentially roosting under the shedding bark of deteriorating snags (Kurta and Kennedy 2002). The lack of permanence in these structures suggests roost fidelity may be more difficult to achieve than that of non-tree roosting species (Gumbert et al. 2002). Further, depletion of snags due to human expansion and unsustainable forest management exerts additional pressures on summer habitat availability for the endangered Indiana bat (Whitaker et al. 2006, Lacki et al. 2007). Increasingly, artificial roosts are used to mitigate destruction of natural roosting cavities, expand habitat availability in managed areas, and facilitate research elucidating the behavior of this threatened bat species (Brittingham and Williams 2000, Rueegger 2016). As deployment of artificial structures has become common practice, understanding factors influencing their use by, and benefit to, bats is imperative to implementing strategic conservation.

Energy conservation is of prime importance during the maternal season, wherein Indiana bat females roost in maternity colonies to birth and rear young until volant (Kurta et al. 1993, Kunz and Lumsden 2003). Although bats use torpor to manage energy expenditures (Hoeh et al. 2018), its use is likely more common in non-reproductive or male bats during the reproductive season, as extended periods of torpor may impede embryo growth and fetal development in pregnant females (Racey and

Swift 1981). Therefore, it is advantageous for bouts of unavoidable torpor to be minimized through selection of warmer roosts, additionally allowing for passive rewarming following torpor (Hoeh et al. 2018). A minimum temperature threshold of 15°C ensures bats maintain stable energy efficiency and arouse from or enter torpor without high energetic costs (Davis and Reite 1967, Wojciechowski et al. 2007), and 15°C has been used as a base measure of roost temperature suitability in previous Indiana and Kentucky-based studies (Tillman 2019, Crawford 2020).

Although solar exposed roosts are preferentially selected (Humphrey et al. 1977, Callahan et al.1997), overheating also poses a threat to roosting bats. Temperatures above 40°C cause heat stress in bats (Licht and Leitner 1967), and colonies avoid temperatures that exceed this threshold by switching roosts (Lourenço and Palmeirim 2004). Instances of fatality occur following overheating events in bat boxes (Flaquer et al. 2014). The collection of artificial roost microclimate data aims to address these concerns, and allows for informed decision-making on roost deployment, while contributing to our understanding of why specific roost sites are selected by bats (Boyles 2007).

Specifically designed to service maternity colonies of Indiana bats, BrandenBark™ artificial roost structures (Copperhead Consulting, Paint Lick, KY) mimic the exfoliating bark found on natural snags and allow bats to move around the interior of the roost for microclimate selection (Hoeh 2017, Gumbert et al. 2013). Roosts constructed of this proprietary material offer consistency of habitat on the landscape, and are used by Indiana bats during maternity season (Adams et al. 2015). My objective was to document temperature profiles and determine habitat suitability for

this form of supplemental roost (hereby referred to as artificial roost) at Veterans Memorial Wildlife Management Area (VWMA), a location with well-documented presence of Indiana bats in Kentucky. I examined temperature differences in roosts selected for, and used intensively, in the maternity season. I hypothesized: i) that temperatures would differ across roosts based on conditions unique to roost clusters found across VWMA (varied placement of roosts in solar exposed and shaded locations within-cluster), as well as at individuals roosts according to microsite differences attributable to aspect (northeast vs. southwest orientation) based on greater solar exposure to the southwest facing side; ii) that live trees would be cooler in temperature than artificial roosts due to live tree canopy cover; iii) and that bats would be found in greater densities at warmer artificial roosts as bats select for greater solar exposure.

STUDY AREA

VWMA was selected as the study site based on a persistent history of maternal use by Indiana bats, coincident with the presence of artificial roosts. Artificial roosts (Fig. 22) used by bats consisted of a 7.6 m utility pole placed at a height of ca. 6.25 m with a layer of polyurethane false bark surrounding the top of the pole (Gumbert et al. 2013). VWMA spans 1,011 ha in the Inner Bluegrass Region (Level IV Ecoregion). Extensive karst is present in the region, which ranges from 152-304 m in elevation, and is characterized by almost level to rolling upland plain topography (Woods et al. 2002). The region's upland forests consist of open forest remnants of oaks and ash, areas of oak-hickory forest and drainages of oak-maple forests. Box elder (*Acer negundo*), tulip poplar (*Liriodendron tulipifera*), black locust (*Robina pseudoacacia*), hackberry (*Celtis*

occidentalis) sweetgum (*Liquidambar styraciflua*), pin-oak (*Quercus palustris*) and mulberry (*Morus*) are common across the region (Woods et al. 2002).

Land use activities surrounding VMWMA include agriculture, suburban development, and horse and livestock pastures. Managed by KDFWR in Scott County, KY (Fig. 2), VWMA supports hardwood forest habitat as well as shrubland, grassland, wetland, and pond habitat (KDFWR 2016). Artificial roosts at VWMA are spread across three distinct areas of the property, allowing for in-depth monitoring of roost inhabitance throughout the site; each roost cluster was regarded as a discrete roost location (Fig. 23). These are referred to as North (n = 6 roosts), Central (n = 7 roosts) and South (n = 6 roost) clusters. Roost clusters are located ca. 0.5-1.5 km from one another while roosts within each cluster are located ca. 30 m apart.

METHODS

Sampling Approach

To record temperatures of artificial roosts, HOBO Pendant Temperature/Light Data Loggers (Onset Computer Corporation, Pocasset, MA) were placed on the exteriors of artificial roost structures (interior placement was not possible due to disturbance concerns in existing structures). Deployment of data loggers occurred when bats were not inhabiting the roost (prior to spring re-inhabitance or after emergence) to minimize disturbance (and with KDFWR and USFWS approval). Data loggers were affixed inside Solar Radiation Shields (Onset Computer Corporation, Pocasset, MA) on either side of a standardized conduit frame (Fig. 24). The frame was placed on top of each roost and secured with wire, with one logger facing the northeast and another the southwest side of each roost structure (n = 38*)*. Data loggers were set to capture

temperature hourly beginning on May 15th, 2019 to gather climatic data associated with the roosts within the maternal study period (Hoeh 2017). Data loggers remained in place through fall to ensure roost inoccupancy upon removal and collect data on fall temperatures for possible use in later studies. A pair of data loggers (oriented northeast and another southwest similar to artificial roosts) was also placed in each cluster on one live shagbark hickory tree (*Carya ovata*), a species used as roost habitat by Indiana bats (Humphrey et al. 1977). Tree loggers ($n = 6$) were affixed using wire at the average height of the other roost loggers within each cluster, and within close proximity (ca. 20 m) to roosts in the North, Central and South clusters.

Bat use of artificial roosts was indexed via standardized guano screens (Fig.22) installed under artificial roosts covering a 1 m² area under the roost (Brigham et al. 2002, Robinson et al. 2019). All roosts were fitted with guano screens, except for roost C3, due to prior installation of an alternate-style guano screen on this roost. Roost C3 was therefore excluded from analysis of guano presence. Guano from each artificial roost was collected late May through mid-July 2019 every 2-3 days from different clusters and counted to generate data on use of specific structures. All guano was cleared from screens following collection.

Data Analysis

Generalized linear models (GLM) approximating a gamma distribution (Table 8) were used in Akaike's Information Criterion (AIC) selection processes (R packages lme4 and AICmodavg, R version 3.6.2, R Core Team 2019) to determine which variables had the greatest influence on daily (24 hour) mean, minimum and maximum roost temperature ($\Delta AIC_c < 2$, Burnum and Anderson 2002). Model parameters included

aspect placement of the loggers (northeast and southwest), cluster location of the roosts (North, Central, South) and seasonality, marked by two-week intervals across the maternity season from May 15th to August 15th to account for changes in temperature as the summer progressed. Model averaging was conducted using R package MuMIN with competing models to identify significant parameters wherein confidence intervals did not cross 0 ((α =0.05), narrowed from earlier analyses due to large sample size). Due to unbalanced sample sizes, a Mann-Whitney U test was conducted (wilcox.test function in base R) for mean, minimum and maximum temperature categories to determine whether temperature profiles differed between artificial roosts and live trees. Finally, a variety of graphing approaches were used to explore relationships between temperature and roost use by bats. Due to priorities of two other research projects during guano collection for bat presence, gaps in the dataset were unavoidable, and guano analysis was limited to the most complete dataset, collected from May 29 - July 7 2019. All means are presented as ± SE and all statistical tests and data visualizations were conducted in RStudio (RStudio Team 2016, R Studio version 1.1.419).

RESULTS

Artificial Roost Temperature

Model selection procedures resulted in two competing models ranked with a $\Delta AIC_c<2$ for mean, minimum and maximum daily temperatures (Tables 9-11, respectively). The cluster and seasonality model and global model accounted for a combined weight of 1.00 in mean, minimum and maximum temperature modeling scenarios. Across response variables, differences in seasonality were influential in determining roost temperature (Tables 12-14). Throughout the maternity season, time

period (defined as bi-weekly intervals from late May to early August) influenced mean, minimum, and maximum daily temperature (Fig. 25-27). Temperature generally increased through the study period, though decreases were notable in early June and late July. Aspect placement of loggers on NE and SW sides of roosts did not cause significant temperature deviations in mean, minimum or maximum daily roost temperature (Fig. 28-30).

North, Central and South roost clusters did not vary significantly from one another in temperature profile. We are unable to interpret the impact of the cluster factor in our model despite the observed output (Tables 12-14) due to the assignment of an incorrect sign in front of parameter estimates for each of the three clusters, an indicator of weak estimates (Gelman and Tuerlinckx 2000, Grueber et al. 2011, Valerie Peters, EKU, pers. communication). When spread across two-week intervals, North cluster temperatures trended slightly higher than Central and South Clusters in mean and minimum daily temperature of roosts, while associating slightly lower with maximum daily temperatures (Fig. 31-33). Central and South clusters trended lower across mean and minimum daily temperature of the roosts, and slightly higher with maximum daily temperature.

Live Tree Temperature

Combined daily mean temperature across the maternity season for artificial roosts was 22.5 ± 0.04°C, while combined daily mean temperature for live trees was 22.4 ± 0.1°C (W=953564, P=0.57). However, daily mean temperature for artificial roosts trended slightly higher than natural roosts throughout (Fig. 34). Mean daily minimum temperature of artificial roosts was 17.4 ± 0.06°C, near to the mean minimum

temperature of live trees 17.6 ± 0.14°C (W=904612, P=0.16). Daily minimum temperatures remained fairly consistent between artificial roosts and natural trees throughout the maternity season, though late May and late June period minimum temperatures extended across a larger range for artificial roosts in comparison to natural roosts (Fig. 35). Mean daily maximum temperature of artificial roosts was 28.3 ± 0.05°C, slightly higher than live trees at 27.8 ± 0.13°C (W=1028959, P<0.01). Artificial roosts consistently reached higher maximum temperatures than live trees throughout the maternity season (Fig. 36).

At the cluster level, the spread of mean daily temperatures was nearly identical for artificial roosts and live trees within North, Central, and South clusters (Fig. 37). The North cluster differed in mean temperature range length by 0.6°C, with artificial roosts ranging from 15.1-27.9°C and live tree temperature ranging from 15.2-27.4°C. Artificial roost mean temperatures ranged from 15.3-27.7°C in the Central cluster, while the live roost tree ranged from 15.3-27.3°C (0.4 °C range difference). Mean temperature range differed for the South cluster by 0.3°C, with artificial roost mean temperature ranging from 15.2-27.0°C and live tree mean temperature ranging from 15.5-27.0°C.

Minimum daily temperatures maintained a consistent range between artificial roosts and natural trees for all three clusters (Fig. 38). The North cluster did not differ in range length for daily minimum temperature. Artificial roosts within the cluster ranged from 8.08-23.6°C, while the live tree ranged from 7.98-23.5°C. The Central cluster differed in minimum range by 1.21°C, with artificial roosts ranging from 6.47-23.2°C and the live tree ranging from 8.08-23.6°C. South cluster roosts differed from the live tree in range by 0.61°C, roosts ranging from 5.45-22.8°C, while the live tree ranged

from 6.06-22.8°C. Maximum daily temperatures differed in range in the North cluster between artificial roosts and live trees by 0.3°C. Artificial roost daily max ranged from 19.2-33.0°C, while the live tree ranged in maximum temperature between 19.4-32.9°C. The Central cluster differed in range between artificial roosts and the live tree by 1.3°C, wherein artificial roosts' daily maximum ranged from 19.5-33.4°C, while live trees ranged from 19.4-32.0°C. The South cluster differed by 0.7°C in daily maximum temperature range between artificial roosts and the live tree. Artificial roost temperatures ranged from 20.5-33.4°C, while the live tree ranged from 20.5-32.7°C (Fig. 39).

Bat Presence

The most guano was collected from the South cluster (21,611 pellets), followed by the Central (13,631 pellets) and North Clusters (4,907) (Fig. 40). All artificial roosts at VWMA saw use during the sampling period. Within each cluster, certain roosts were favored (Fig. 41). Specifically, roosts N3, C5, and S5 were identified as the greatest-use roosts at each cluster, and cumulatively accounted for 36% of all guano collected in the study. Individually, roost N3 accounted for 44% of guano collected at the North cluster, C5 for 34% at the Central cluster, and S5 for 36% at the South cluster. At higher mean temperatures, bats did not noticeably favor a particular cluster (Fig. 42). Even so, the colony was spread most evenly at highest recorded temperature periods. Earlier in the season, bats favored the South cluster, followed by the Central cluster (Fig. 43). Guano at the Central and North clusters increased in presence throughout the season, while guano at the South cluster leveled off as the colony spread out within the WMA.

DISCUSSION

Roost temperatures varied as expected during the maternity period, generally warming following early June. Despite the importance of solar exposure for Indiana bat maternity roosts (Britzke et al. 2003), aspect was an unexpectedly insignificant parameter in predicting roost temperature. Microclimatic variation within roosts allows bats to behaviorally thermoregulate without exiting the roost and risking predation (Terrien 2011), and it is reasonable to assume that Indiana bats exhibited such behavior at VWMA (Crawford 2020).VWMA offers mixed placement of artificial roosts within each cluster in open and shaded locations, which would allow bats some level of selection across variable roost temperatures. Despite common placement across both forested and exposed locations, the external air temperature of the roost did not differ enough to be significant in this scenario, counter to my hypothesis that southwest facing roosts would be warmer. I expect placement of loggers in the interior of artificial roosts may have yielded more dramatic results, based on the assumption that heat would be trapped by the polyurethane bark, as western sides of these roosts are warmer (Hoeh 2017). Counter to expectations, roost clusters did not differ in overall mean, minimum or maximum daily temperatures.

The highest temperature events ($n = 4$) throughout our monitoring season were recorded in late July, wherein four separate artificial roosts (1 located in the Central cluster, 3 in the South) reached 33.43°C. No temperature measurements were collected in our study period that met or exceeded the maximum temperature threshold of 40°C, a temperature reported in the literature as a critical upper limit for bats (Licht and Leitner 1967). Our exterior data logger placement may have impacted these results, as a study

in Indiana (ca. 200 miles from our study site) recorded artificial roost internal temperatures reaching 60°C (Hoeh 2017). While we cannot confirm internal roost temperatures in our study, it appears roosts falling below the 15°C minimum threshold of temperature stability (observed in Davis and Reite (1967) and used by Tillman (2019) and Crawford (2020) in similar studies in the region) are the greater threat to artificial structure roosting bats at VWMA.

The lowest recorded temperature during our survey period was 5.45°C, logged in early June on both aspects of artificial roost S5 in the South cluster. The daily minimum temperature for individual loggers fell below the minimum threshold on 648 instances across 29 days, representing temperatures across all roosts. Although the interiors of roosts provide enclosed spaces for warming, it is likely that many of these instances mirror temperatures below the minimum threshold within roosts. As captures in our study consisted primarily of reproductive females, it is clear that VWMA artificial roosts are used as maternity colony habitat. Inhabiting roosts at these temperatures may require females to expend excess energy on warming and arousal from torpor, impacting maternal development and potentially leading to slower pup growth in cooler temperatures (Hoying and Kunz 1998). These observations are of concern for managers, as the supplementation of maternal habitat for an endangered bat species must facilitate adequate conditions for pup survival and growth.

Counter to expectation that artificial roosts would be warmer than live trees, live tree temperatures presented similarly to artificial roosts. The highest recorded temperature for a live tree reached 32.90°C, and no instances were recorded above the maximum threshold. The coolest instance recorded at a live tree was 6.06°C, with 90

instances where the minimum temperature dropped below 15°C (Davis and Reite 1967, Tillman 2019, Crawford 2020). Despite similar thermal extremes between artificial roosts and live trees assessed in the study, artificial roosts appear to be more variable than live trees throughout the season. While there were far more observations for artificial roosts in comparison to live trees, these observations are a useful reference. Based on persistent roost use, and comparable temperature profiles to natural trees, artificial roosts serve as a functional replacement for natural roost trees at VWMA.

The lack of significant temperature differences between clusters, and the common, persistent use of all roost clusters at VWMA indicates each provides suitable habitat for Indiana bat maternity colonies. The most intensely inhabited of the three clusters, the South cluster, seemingly had the most preferential suite of roosts due to a more even spread of use across artificial roosts within the cluster. Indiana bat maternity colonies preferentially inhabit bottomlands (Carter 2006). Although the elevation change is minimal (ca. 35 meters) between the North and South sites, the small valley the South cluster resides in likely offers wind shelter while still maintaining open flyway (Fig. 23). The South corridor is directly along a stream at VWMA, notable due to a restoration and native riparian planting that occurred there prior to the onset of my project (Sustainable Streams, LLC 2018).

Supporting the second-greatest amount of use, the Central cluster is situated ca. 10 m higher in elevation than the South cluster, and ca. 25 m lower than the North cluster. The Central cluster is the most open of the three areas, within immediate access to a large pond. Least preferential of the three sites, the North cluster's lesser use may be due to its position as the only cluster atop a ridge and entirely surrounded by forest

(Fig. 23). The sheltered layout was expected to be favorable to Indiana bats for predator avoidance and foraging, as they are slow flying, short-winged and, therefore, a clutter-adapted bat species (Norberg and Rayner 1987). Lower counts within the North cluster are also likely associated with the fewer collection days able to be conducted there, due to priorities of a secondary project.

Given proximity of the Central and South clusters (<0.5 km apart), bats are likely interchanging between the sites more frequently. Remaining in either cluster provides access to a larger array of roosts, while reaching the northern roost cluster requires movements of ca. 0.9 km or 1.4 km (Central and South distances to North, respectively, Fig. 23). Although distances are well within female Indiana bat home ranges during the maternal season (Womack et al. 2013), pregnant and lactating individuals likely exhibited fidelity to established roosts due to bodily stress and pup care (Humphrey et al. 1997, Womack et al. 2013). While use skewed most heavily to the South site at the beginning of the maternity period, I speculate that the more even spread across all three sites later in the season may indicate the population breaking into smaller colonies to rear young in key roosts within each cluster. Expansion likely increased as pups became volant and relocated from the maternity roost into other available roosts and clusters. Spread of the colony throughout VWMA is advantageous for protection of bats in case of adverse weather or other unexpected events, and further emphasizes the importance of multiple roost placements across varied micro-habitats.

Counter to expectations, bats were not found in greatest densities at warmer artificial roosts. Within the heavily occupied South cluster, roost S5 was the most intensively used artificial roost at VWMA in 2019. S5 remained in direct solar exposure

and stood apart from surrounding vegetation. S6, the second-greatest use roost within the South cluster, contrasted with S5 given its proximity to surrounding tree cover, though external mean temperatures between the two did not vary across the two time intervals in June (20.9 ± 0.53°C, 20.9 ± 0.53°C) a key period for pregnancy and parturition. In comparison, the two lowest use roosts in the South cluster, S2 and S3, presented with near identical mean temperatures to the greatest use roosts during the two focal time intervals in June (20.8 ± 0.35°C, 20.9 ± 0.35°C). The greatest use roosts within the other two clusters also followed the overall solar exposure pattern of the two South cluster roosts, but contrasted in solar condition of the greatest use roost. Roost C4 in full sun was the second-greatest use roost within the Central cluster, in comparison to C5 in partial shade, while the North cluster's partially shaded N3 was the greatest use roost in comparison to N4 in full sun. Although external mean temperature did not vary during the two week measurement periods in June between C4 and C5 (21.2 ± 0.34°C, 21.2 ± 0.34°C) or N3 and N4 (21.3 ± 0.34°C, 21.4 ± 0.34 °C), movement of bats within a cluster between two suitable roosts is indicated by these results. Roosts C4 and C5 in the Central cluster did not differ from the least used roosts in the cluster C6 and C7 (21.1 ± 0.34°C, 21.1 ± 0.34°C). N2 and N5, the lowest use roosts in the North cluster, also did not differ within the two June time intervals from the greatest use roosts in the same cluster (21.4 ± 0.34°C, 21.4 ± 0.49°C), roosts N3 and N4. Future placement of internal loggers may also reveal deviation in microclimate that impacted roost selection and movement in the three clusters.

It is notable that all artificial roosts within VWMA were used to varying degrees in 2019. Additionally, roost switching was confirmed during the summer maternity

period via recaptures of bats within the season (see Chapter 2). This indicates the suitability of roosts placed in a variety of habitat conditions, and the ability of bats to successfully relocate if temperatures at a single roost are unsuitable, or as colony sizes increase as pups are added to the population. Despite variation in temperature and habitat, pregnant, lactating or juvenile bats were observed in North, Central and South clusters, evidencing the suitability of each for reproduction and pup rearing. Also, given fluctuations in roost occupancy over short (several day) periods, it is likely that factors other than temperature impact roost selection by Indiana bats.

MANAGEMENT IMPLICATIONS

Maternity habitat is critical for pup survival, and vital for management of an endangered species threatened by habitat loss and the spread of WNS. Studies on the use of artificial habitat enhancement structures support effective allocation of resources by wildlife management agencies. Artificial roosts serve as a practical tool to allow periodic monitoring of Indiana bats due to their persistence on the landscape and consistent use by bats. However, roosts should be inspected regularly for rot and overall degradation to ensure the safety of both humans and bats. It would benefit future studies at VWMA to monitor temperatures between artificial roosts and natural snags across the landscape to more accurately compare maternity habitats for the Indiana bat. Additionally, studies focused on other potential factors in roost selection (parasite loads, proximity to water, distance to cover, etc.) should be conducted to elucidate proper placement and management of artificial roosts for Indiana bats.

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APPENDICES

Appendix A: Tables

Appendix A: Tables

Table 1. Model parameters used in Akaike's Information Criterion (AIC) model selection to determine influential factors on adult female Indiana bat mass.

Model	Model Type	Factors	Code
1	Null	Julian date Site	~1 + (1|Julian) + (1|site)
2	Body morphometric	Reproductive condition Right forearm length Julian date Site	reprocdt + RFA + (1|Julian) + (1|site)
3	WNS wing score and body morphometric	Wing score Reproductive condition Right forearm length Julian date Site	wingscore + reprocdt + RFA + (1|Julian) + (1|site)
4	WNS year and body morphometric	Year Reproductive condition Right forearm length Julian date Site	year + reprocdt + RFA + (1|Julian) + (1|site)

5	WNS wing score only	Wing score Julian date Site	wingscore + (1|Julian) + (1|site)
6	WNS year only	Year Julian date Site	year + (1|Julian) + (1|site)
7	Global	Year RFA Reproductive condition Wing score Julian date Site	year + RFA + reprocdt + wingscore + (1|Julian) + (1|site)

Table 2. Indiana bat captures per netting event organized by study site. (-) indicates site not surveyed.

Study Site	2017		2018		2019	
	June	July	June	July	June	July
BARF	17	-	25	33	52	22
VWMA Central	-	-	63	-	46	-
VWMA South	39	-	34	-	51	-
VWMA North	62	18	26	48	23	43
YWMA	10	63	52	46	59	34
Total	128	81	200	127	231	99

Table 3. Model selection results ranked by Akaike's Information Criterion (AIC) to determine influential factors on adult female Indiana bat mass. Competing models indicated in bold.

Model	*K*	*df*	ΔAIC_c	w_i
WNS year and body morphometric	**10**	**537**	**0.00**	**0.41**
Body morphometric	**8**	**539**	**0.69**	**0.29**
WNS wing score and body morphometric	**10**	**537**	**1.83**	**0.16**
Global	12	535	2.11	0.14
WNS year only	6	541	389.93	0.00
Null	4	543	405.53	0.00
WNS wing score only	6	541	406.49	0.00

Table 4. Model averaged estimates of explanatory variables for post-WNS adult female mass models. Estimates in bold indicate significance (α = 0.15).

Variable	Estimate	SE	85% CI Lower Limit	85% CI Upper Limit
Intercept	0.928	0.336	0.443	1.413
Year 2017 (RefLvl: 2018)	-0.027	0.020	-0.055	0.002
Year 2018	0.027	0.020	-0.002	0.055
Year 2019	-0.007	0.019	-0.035	0.020
Year 2019* (RefLvl:2018)	**-0.034**	**0.016**	**-0.057**	**-0.011**
Right forearm length	**0.274**	**0.054**	**0.196**	**0.352**
Repro cdt. lactating (RefLvl: non-repro.)	-0.008	0.019	-0.035	0.019
Repro cdt. non-repro.	0.008	0.019	-0.019	0.035
Repro cdt. pregnant	**0.345**	**0.014**	**0.324**	**0.365**
Repro cdt. post-lac.	-0.024	0.023	-0.058	0.010
Wing score 0 (RefLvl: 1)	**0.022**	**0.012**	**0.004**	**0.039**
Wing score 1	**-0.022**	**0.012**	**-0.039**	**-0.004**
Wing score 2	-0.004	0.096	-0.141	0.134

***Year 2019 is listed twice to depict significant result when compared with reference level 2018. Initial listing is reference level 2017.

Table 5. Bat captures (all species) per netting event organized by study site. (-) indicates site not surveyed.

Study Site	2017		2018		2019	
	June	July	June	July	June	July
BARF	19	-	25	33	53	22
VWMA Central	-	-	63	-	46	-
VWMA South	39	-	34	-	51	-
VWMA North	62	18	26	48	23	43
YWMA	10	63	53	46	59	34
Total	130	81	201	127	232	99

Table 6. Total bat recaptures across years and sites (-) indicates site not surveyed.

Study Site	2017		2018		2019		Total	
	n	%	n	%	n	%	n	%
BARF	1	1	14	12	22	18	37	30
VWMA North	1	1	14	12	7	6	22	18
VWMA Central	-	-	18	15	8	7	26	22
VWMA South	0	0	5	4	9	8	14	12
YWMA	0	0	9	8	11	9	20	17

Table 7. Instances of overlap (recapture rate %) between North, Central and South roost cluster sites for bats banded at Veterans Memorial Wildlife Management Area that were recaptured at the same or another cluster.

	Recapture rate (%)		
Site of initial banding	Site of second capture		
	North	Central	South
North	7	8	2
Central	1	3	4
South	7	9	5

Table 8. Model parameters used in Akaike's Information Criterion (AIC) model selection to determine influential variables for roost temperature. Model format used for mean, minimum, and maximum temperature model selection.

Model	Model Type	Factors	Code
1	Null		~1
2	Cluster and seasonality	Cluster Seasonality	cluster + seasonality
3	Aspect and seasonality	Aspect Seasonality	aspect + seasonality
4	Cluster and aspect	Cluster Aspect	cluster + aspect
5	Global	Cluster Seasonality Aspect	cluster + seasonality + aspect

Table 9. Akaike's Information Criterion (AIC) model ranking results for mean temperature of roosts. Competing models indicated in bold.

Model	*K*	*df*	ΔAIC_c	w_i
Cluster and seasonality	**9**	**3396**	**0.00**	**0.71**
Global	**10**	**3395**	**1.81**	**0.29**
Aspect and seasonality	8	3397	41.85	0.00
Cluster and aspect	5	3400	1542.48	0.0
Null	2	3403	1564.95	0.00

Table 10. Akaike's Information Criterion (AIC) model ranking results for minimum temperature of roosts. Competing models indicated in bold.

Model	*K*	*df*	ΔAIC_c	w_i
Cluster and seasonality	**9**	**3396**	**0.00**	**0.72**
Global	**10**	**3395**	**1.93**	**0.28**
Aspect and seasonality	8	3397	108.46	0.00
Cluster and aspect	5	3400	855.07	0.0
Null	2	3403	936.53	0.00

Table 11. Akaike's Information Criterion (AIC) model ranking results for maximum temperature of roosts. Competing models indicated in bold.

Model	*K*	*df*	ΔAIC_c	w_i
Cluster and seasonality	**9**	**3396**	**0.00**	**0.70**
Global	**10**	**3395**	**1.65**	**0.30**
Aspect and seasonality	8	3397	31.12	0.00
Cluster and aspect	5	3400	1611.28	0.0
Null	2	3403	1626.60	0.00

Table 12. Model averaged estimates of explanatory variables for mean temperature models. Estimates in bold indicate significance ($\alpha = 0.05$).

Variable	Estimate	SE	95% CI Lower Limit	95% CI Upper Limit
Intercept	0.043	0.000	0.043	0.044
Aspect NE (RefLvl: SW)	0.000	0.000	0.000	0.000
Aspect SW	0.000	0.000	0. 000	0.000
North cluster*	0.000	0.000	-0.001	0.000
Central cluster* (RefLvl: North)	0.000	0.000	0.000	0.001
South cluster*	0.001	0.000	0.000	0.001
Late May	**0.003**	**0.000**	**0.003**	**0.004**
Early June	**0.008**	**0.000**	**0.007**	**0.008**
Late June	**0.001**	**0.000**	**0. 000**	**0. 001**
Early July	**-0.003**	**0.000**	**-0.003**	**-0.002**
Late July	**0.000**	**0.000**	**-0. 001**	**0.000**
Early August (RefLvl: Late May)	**-0.003**	**0.000**	**-0.004**	**-0.003**

* Denotes model assignment of an incorrect sign in front of parameter estimates, an indicator of weak estimates (Gelman and Tuerlinckx 2000, Grueber et al. 2011).

Table 13. Model averaged estimates of explanatory variables for minimum temperature models. Estimates in bold indicate significance ($\alpha = 0.05$).

Variable	Estimate	SE	95% CI Lower Limit	95% CI Upper Limit
Intercept	0.056	0.000	0.055	0.057
Aspect NE (RefLvl: SW)	0.000	0.000	-0.001	0.001
Aspect SW	0.000	0.000	-0. 001	0.001
North cluster*	-0.002	0.000	-0.002	-0.001
Central cluster* (RefLvl: North)	0.002	0.000	0.001	0.002
South cluster*	0.003	0.000	0.002	0.004
Late May	**0.004**	**0.001**	**0.002**	**0.005**
Early June	**0.014**	**0.001**	**0.012**	**0.015**
Late June	**-0.001**	**0.001**	**-0.002**	**0.000**
Early July	**-0.006**	**0.001**	**-0.007**	**-0.005**
Late July	**-0.003**	**0.001**	**-0.004**	**-0.002**
Early August (RefLvl: Late May)	**-0.004**	**0.001**	**-0.005**	**-0.002**

* Denotes model assignment of an incorrect sign in front of parameter estimates, an indicator of weak estimates (Gelman and Tuerlinckx 2000, Grueber et al. 2011).

Table 14. Model averaged estimates of explanatory variables for maximum temperature models. Estimates in bold indicate significance ($\alpha = 0.05$).

Variable	Estimate	SE	95% CI Lower Limit	95% CI Upper Limit
Intercept	0.034	0.000	0.034	0.034
Aspect NE (RefLvl: SW)	0.000	0.000	0.000	0.000
Aspect SW	0.000	0.000	0.000	0.000
North cluster*	0.000	0.000	0.000	0.000
Central cluster* (RefLvl: North)	0.000	0.000	0.000	0.000
South cluster*	-0.001	0.000	-0.001	0.000
Late May	**0.003**	**0.000**	**0.002**	**0.003**
Early June	**0.006**	**0.000**	**0.006**	**0.007**
Late June	**0.001**	**0.000**	**0.001**	**0.002**
Early July	**-0.001**	**0.000**	**-0.002**	**-0.001**
Late July	**0.000**	**0.000**	**0.000**	**0.001**
Early August (RefLvl: Late May)	**-0.003**	**0.000**	**-0.003**	**-0.002**

* Denotes model assignment of an incorrect sign in front of parameter estimates, an indicator of weak estimates (Gelman and Tuerlinckx 2000, Grueber et al. 2011).

Appendix B: Figures

Appendix B: Figures

Figure 1. BrandenBark™ artificial roost structure at Bernheim Arboretum and Research Forest with mesh guano catch installed below.

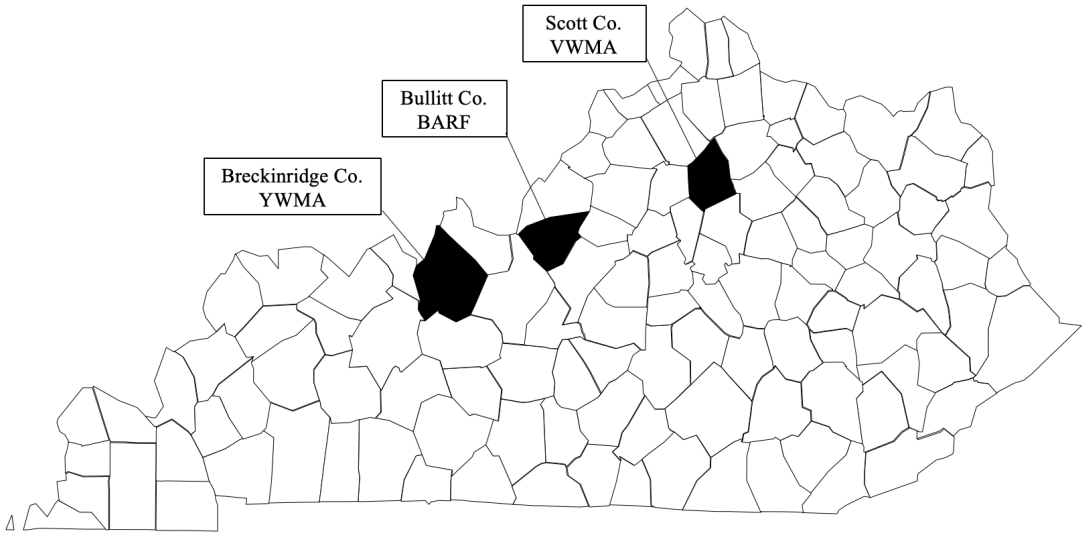

Figure 2. Study site counties.

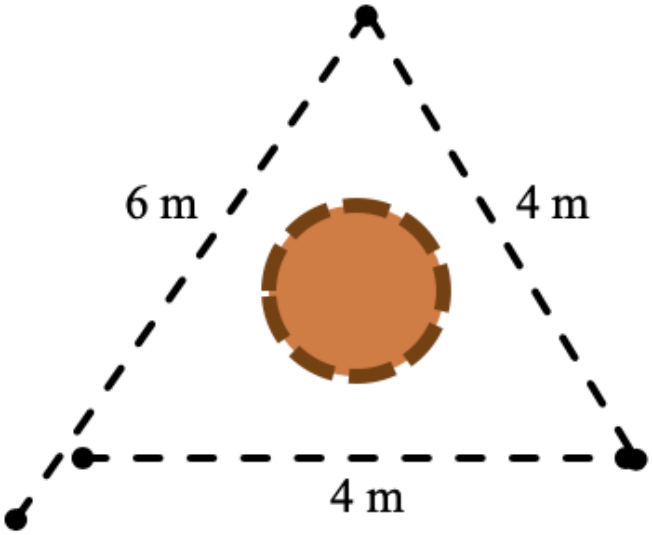

Figure 3. Mist net triangle formation surrounding artificial roost structure.

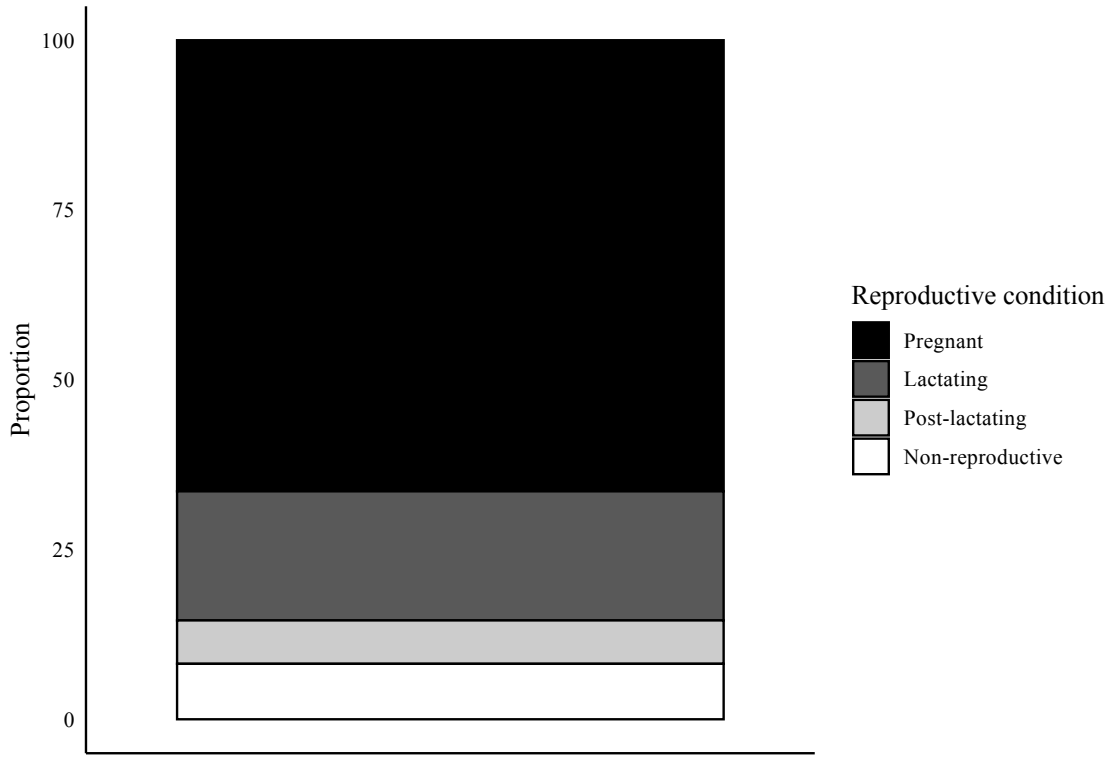

Figure 4. Proportion of adult female Indiana bat captures in each reproductive class (*n* = 547).

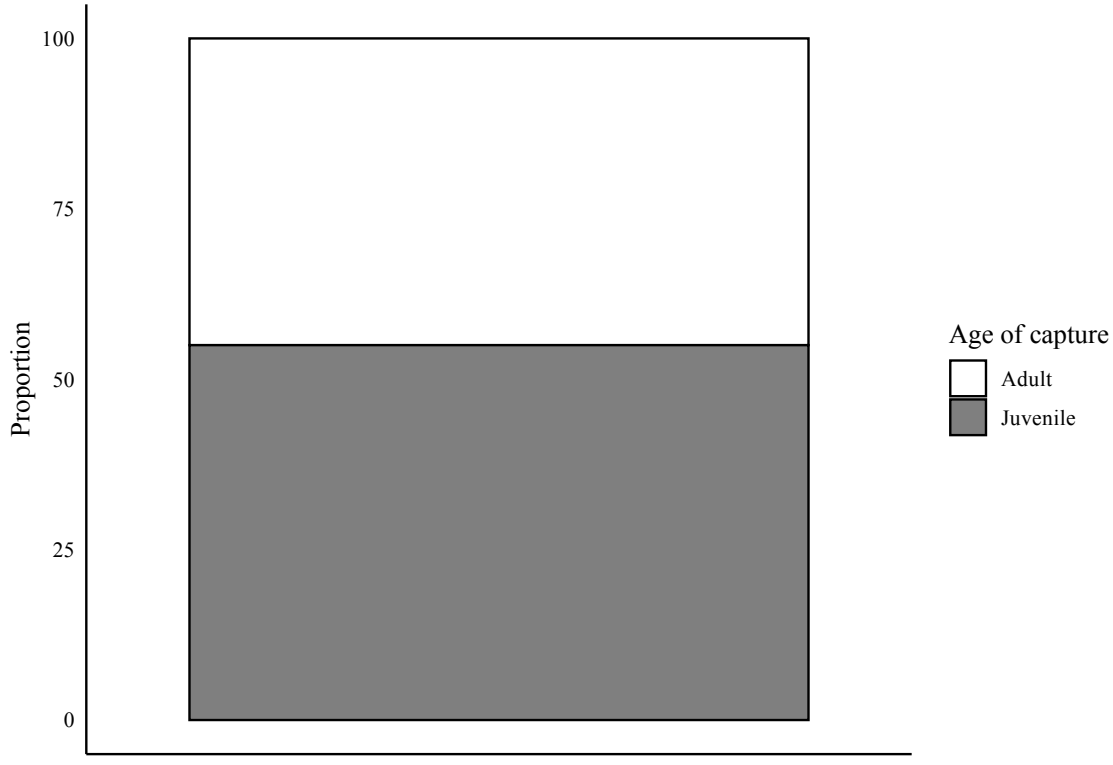

Figure 5. Age demographics of total July captures of Indiana bats (*n* = 168).

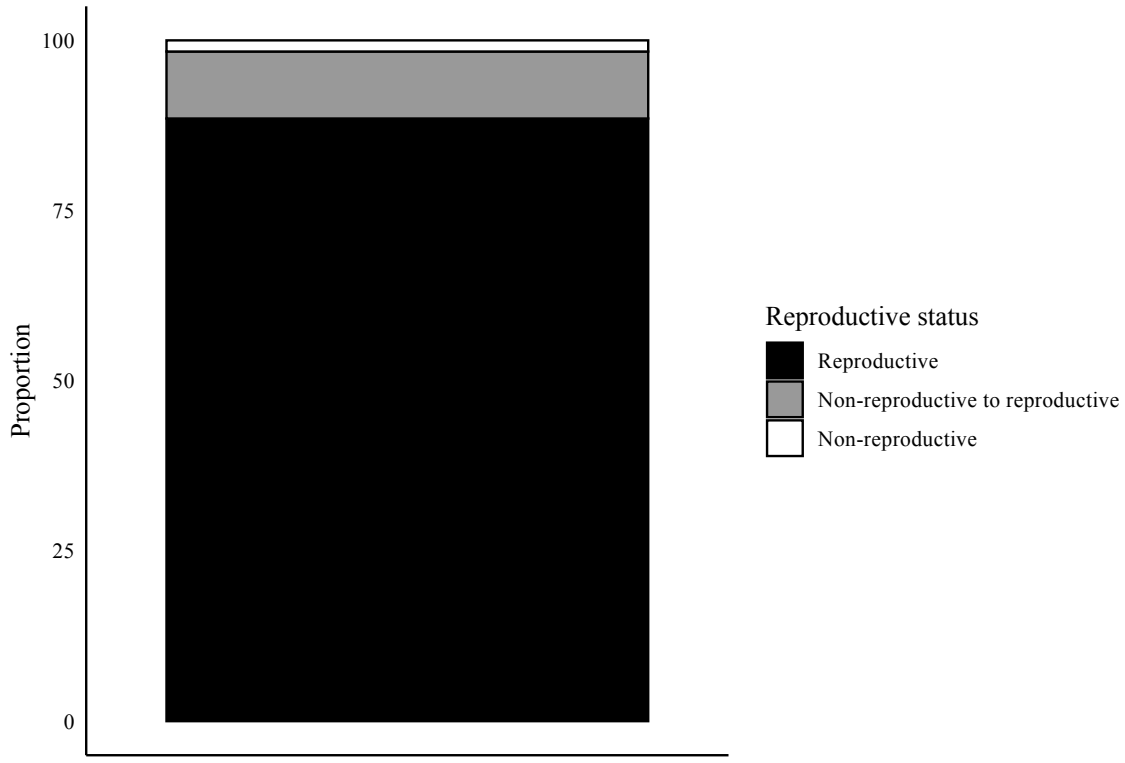

Figure 6. Reproductive status across two capture instances for adult female Indiana bat recaptures (n = 61). Individuals were either reproductive or non-reproductive at both captures, or changed status from non-reproductive to reproductive between captures.

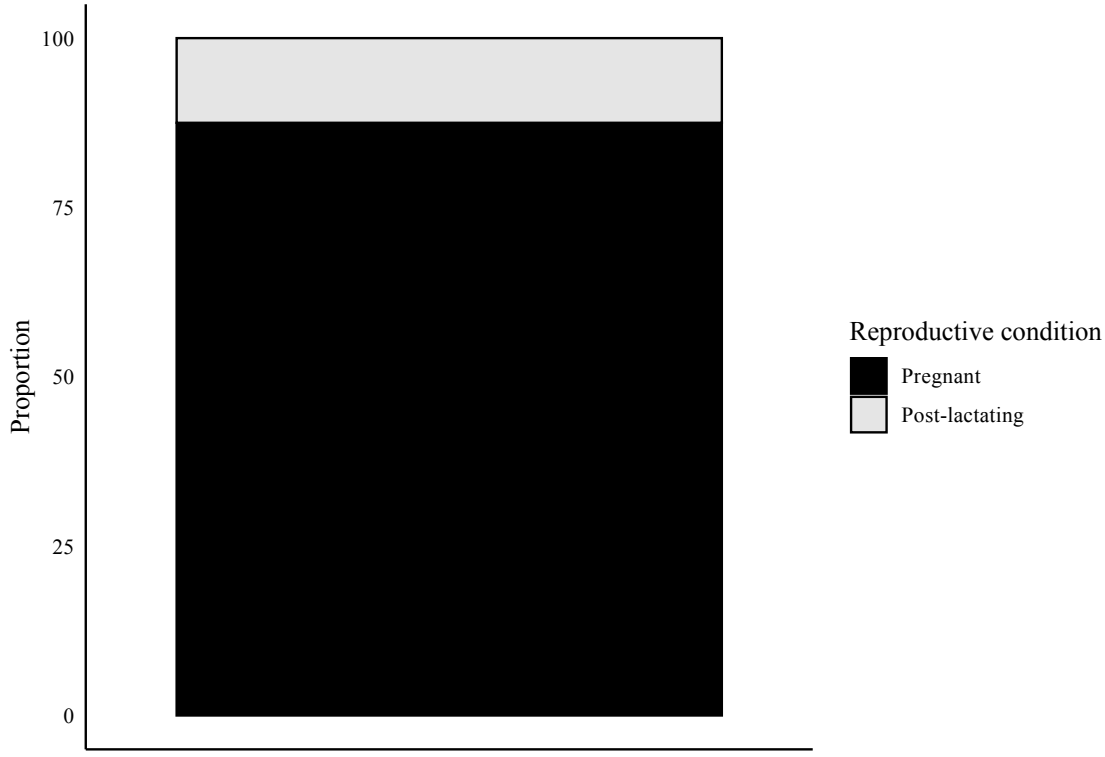

Figure 7. Reproductive status of adult female Indiana bat recaptures initially captured as juveniles (n = 8).

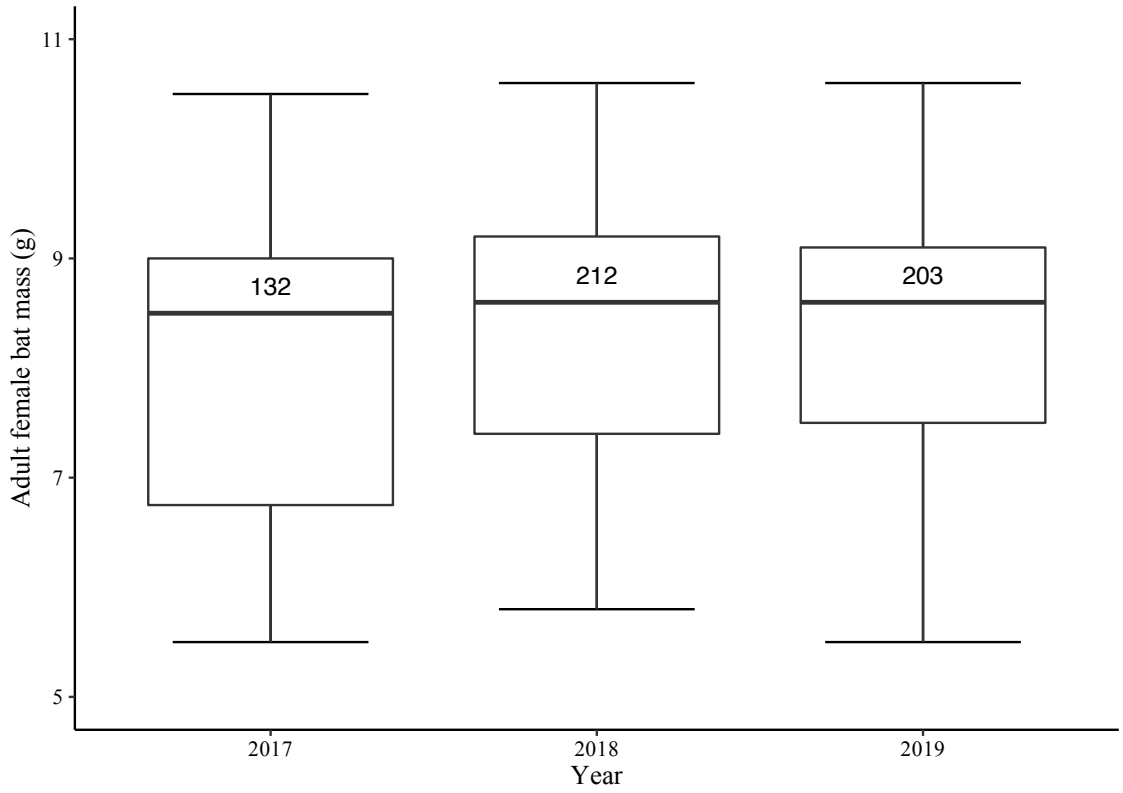

Figure 8. Boxplot of adult female Indiana bat mass by year of post-WNS capture group. Sample size is listed above each median.

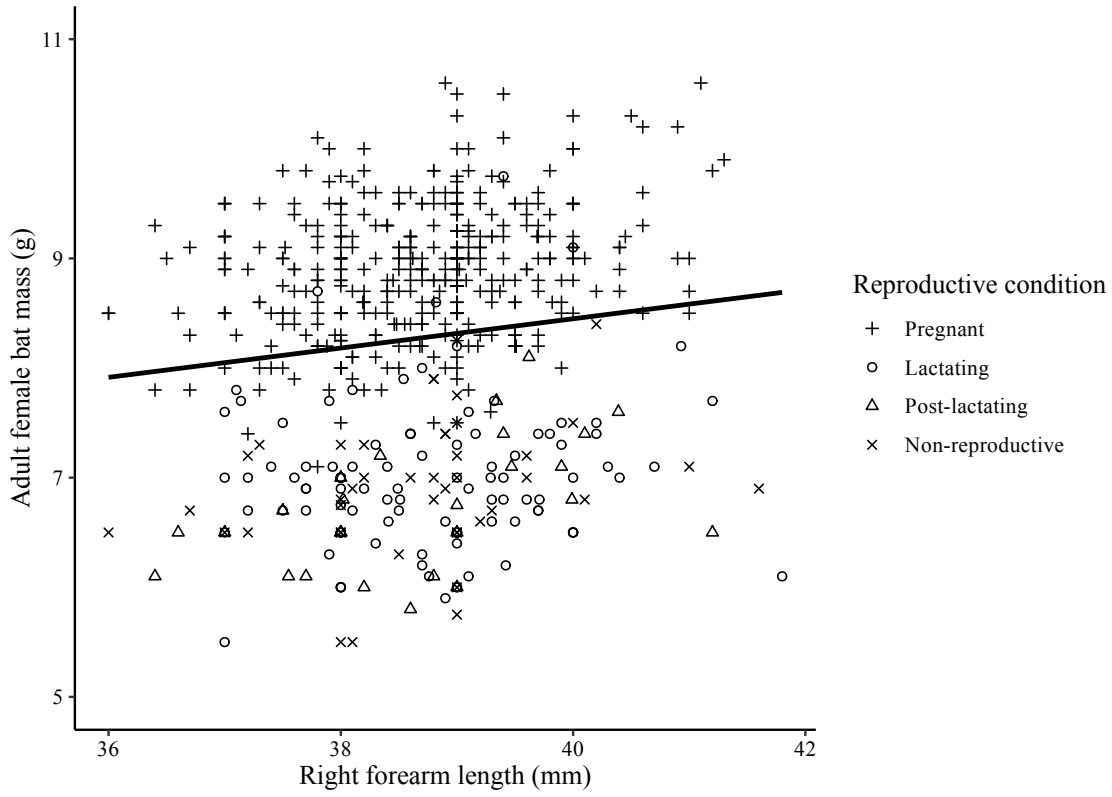

Figure 9. Relationship between Indiana bat mass and forearm length characterized by reproductive condition.

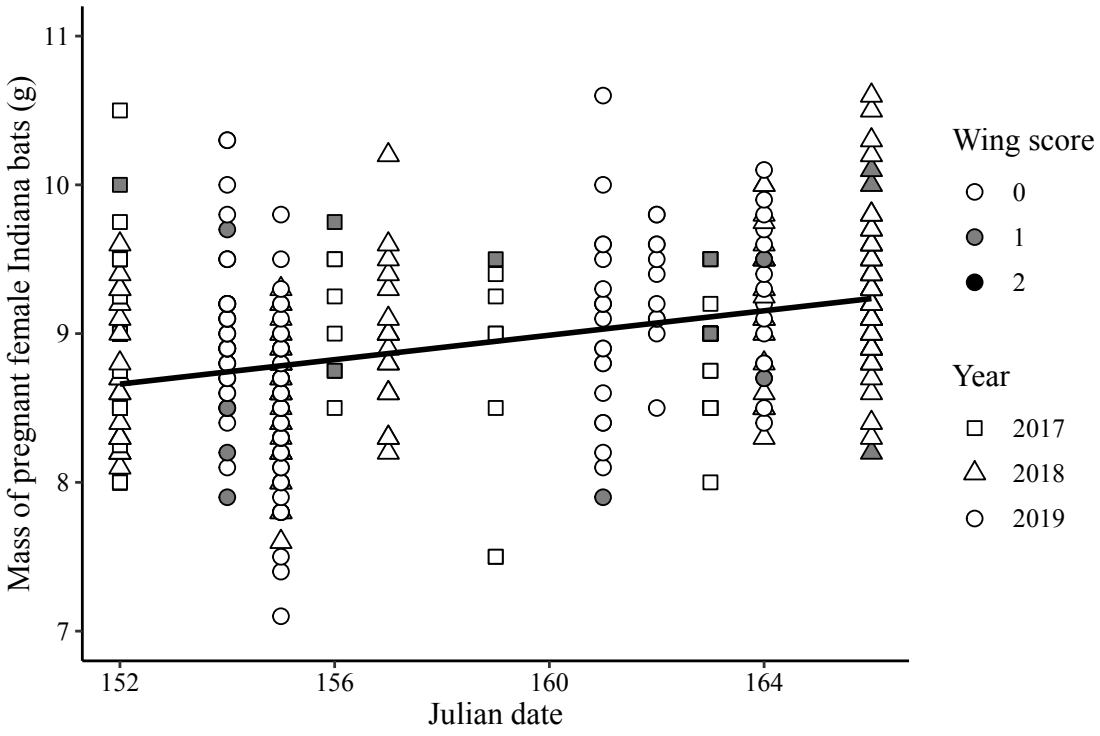

Figure 10. Distribution of wing score in relation to mass of pregnant Indiana bats organized by Julian date.

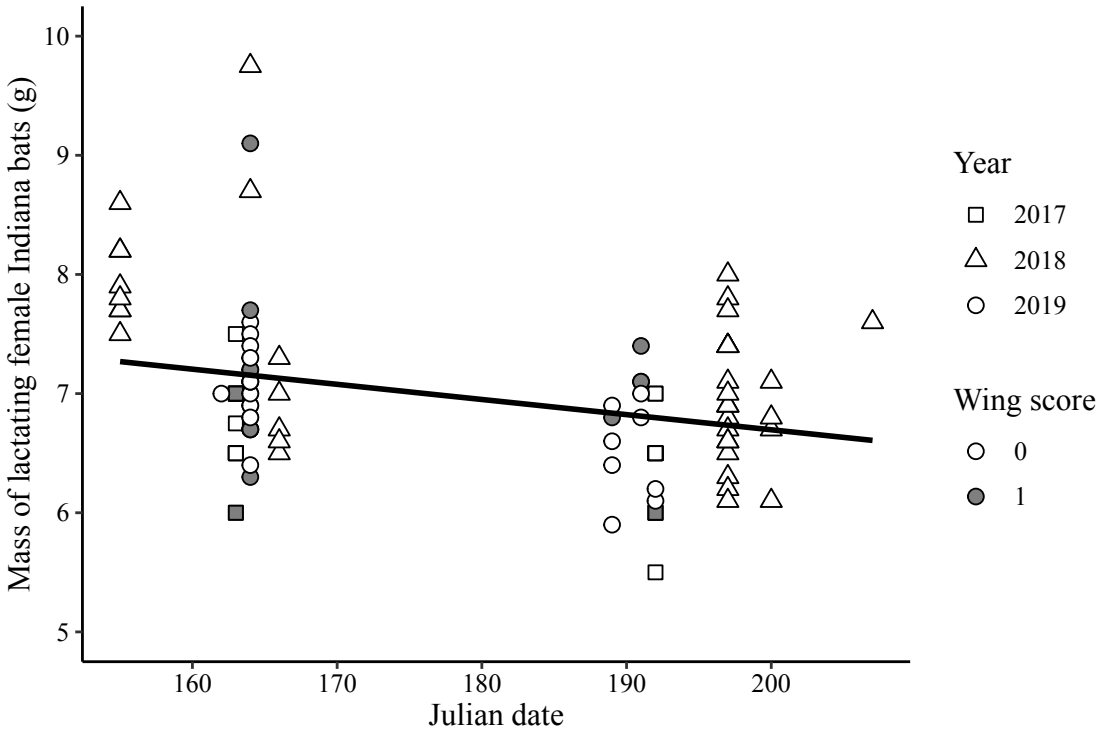

Figure 11. Distribution of wing score in relation to mass of lactating Indiana bats organized by Julian date.

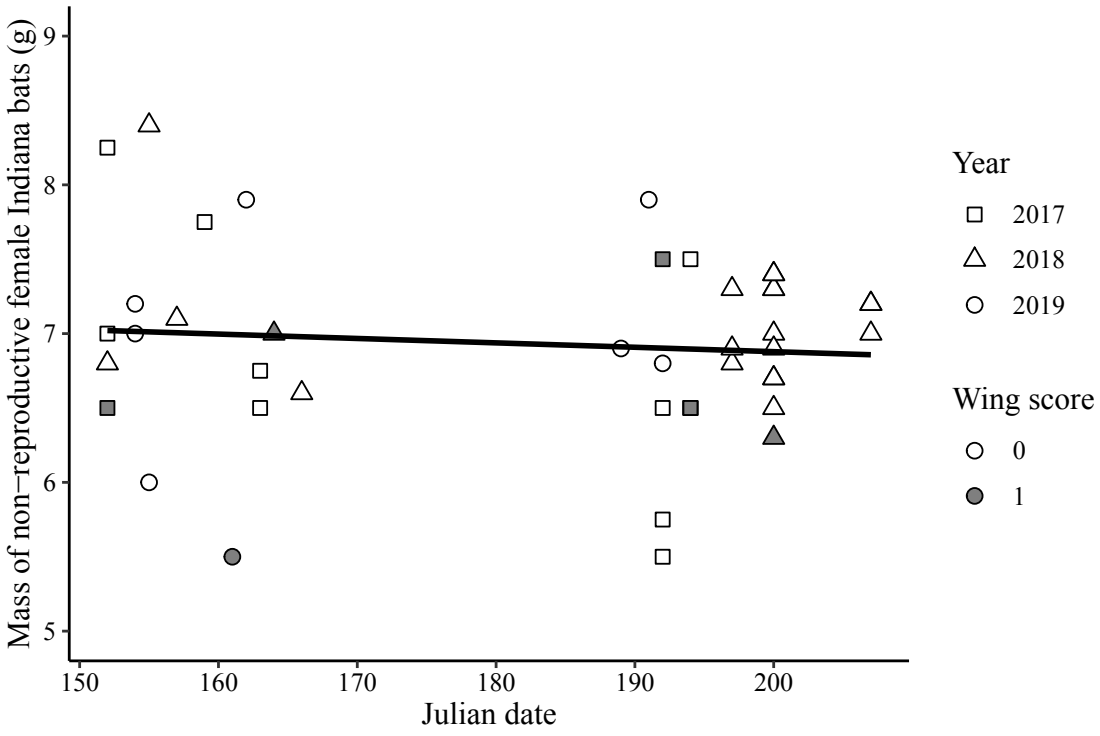

Figure 12. Distribution of wing score in relation to mass of non-reproductive Indiana bats organized by Julian date.

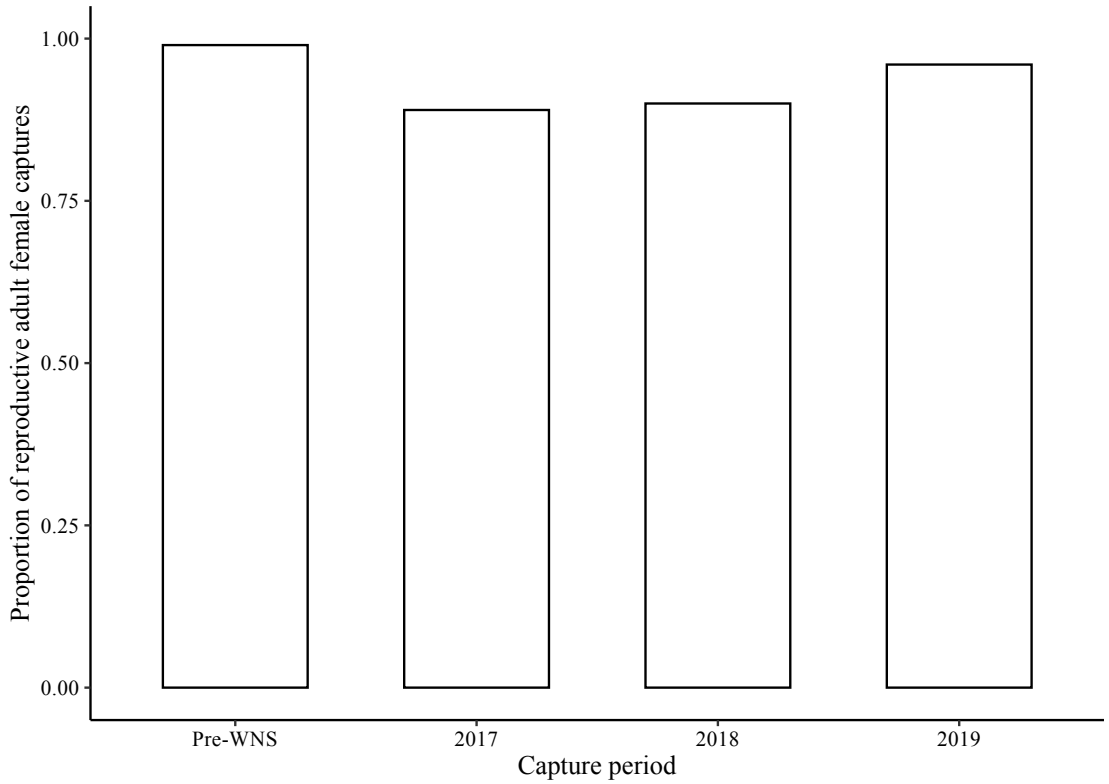

Figure 13. Proportion of adult female Indiana bats in the reproductive population within the pre-WNS dataset in comparison to individual post-WNS years that were part of this study.

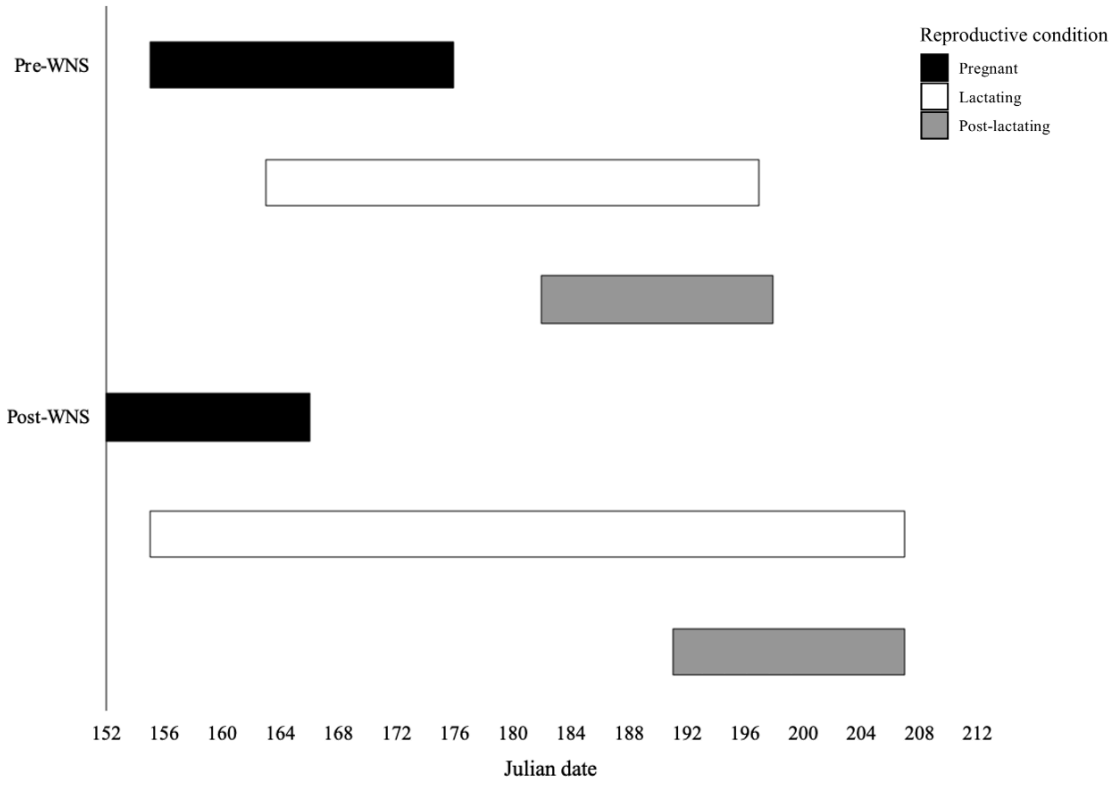

Figure 14. Reproductive time periods categorized by reproductive class, measured by Julian day. Post-WNS sampling term began at Julian date 152.

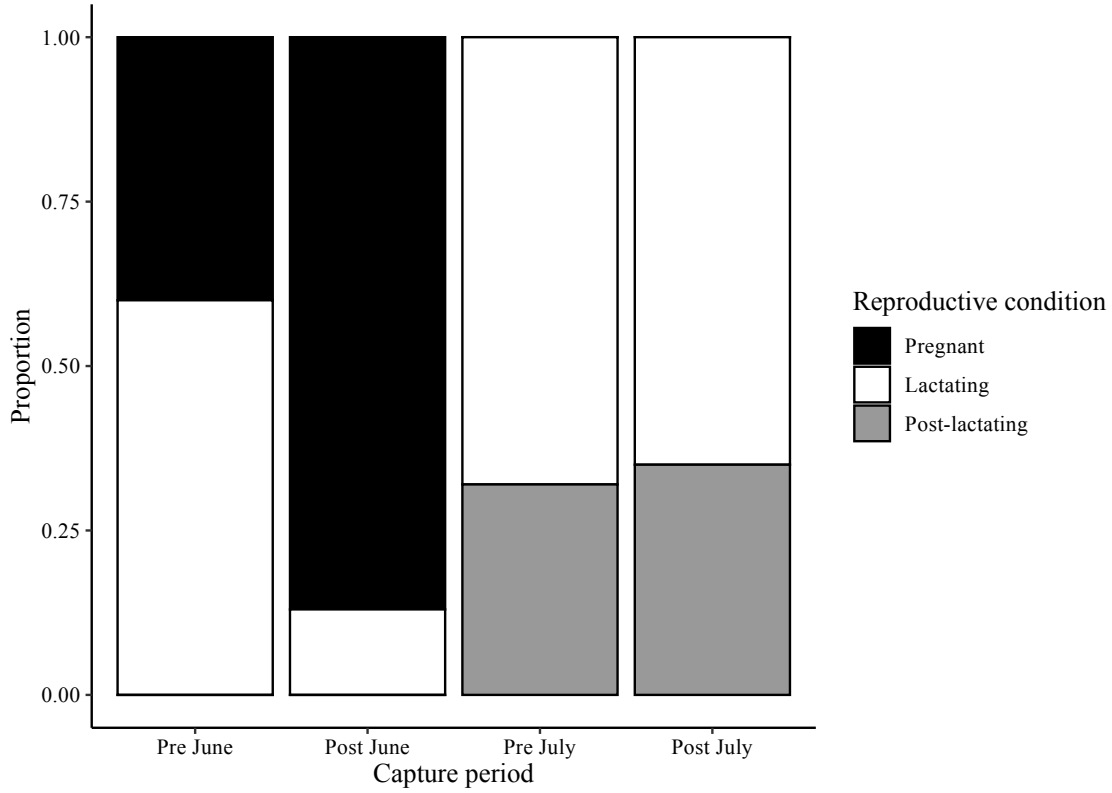

Figure 15. Proportion of Indiana bats in each reproductive class during June and July for pre and post-WNS capture populations.

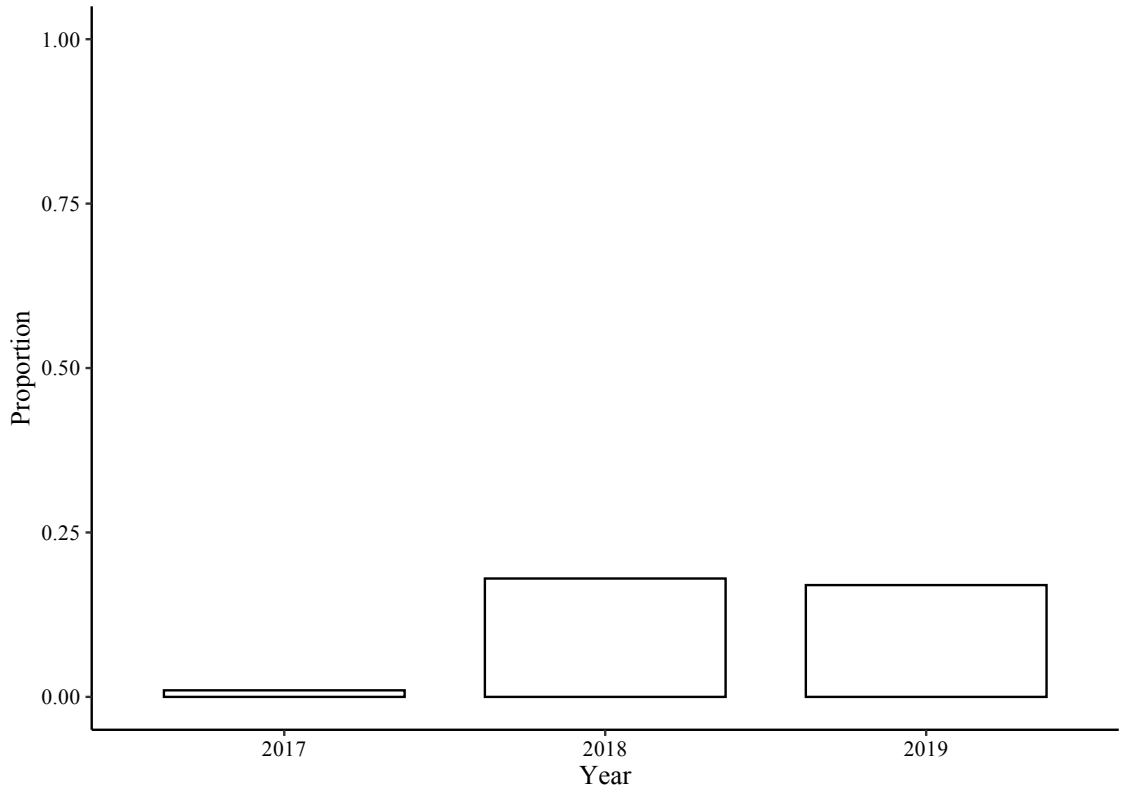

Figure 16. Proportion of total bat captures collected as recaptures within each year.

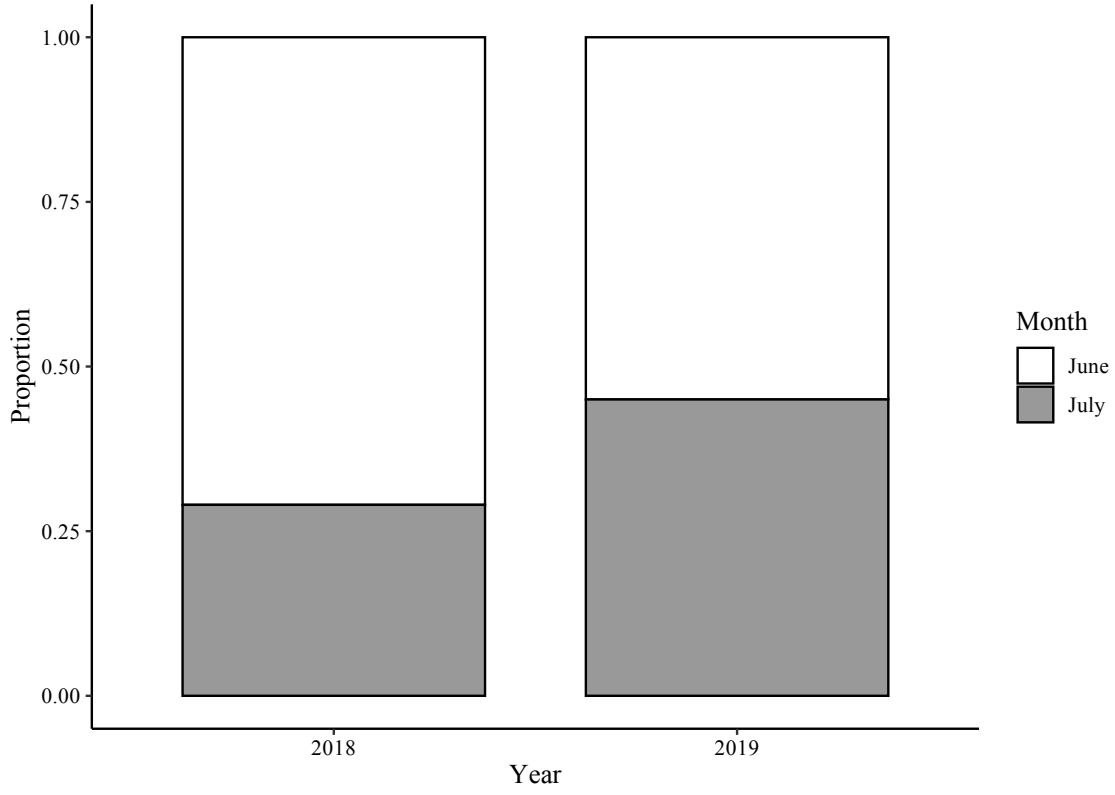

Figure 17. Within-year recapture occurrences at Bernheim Arboretum and Research Forest.

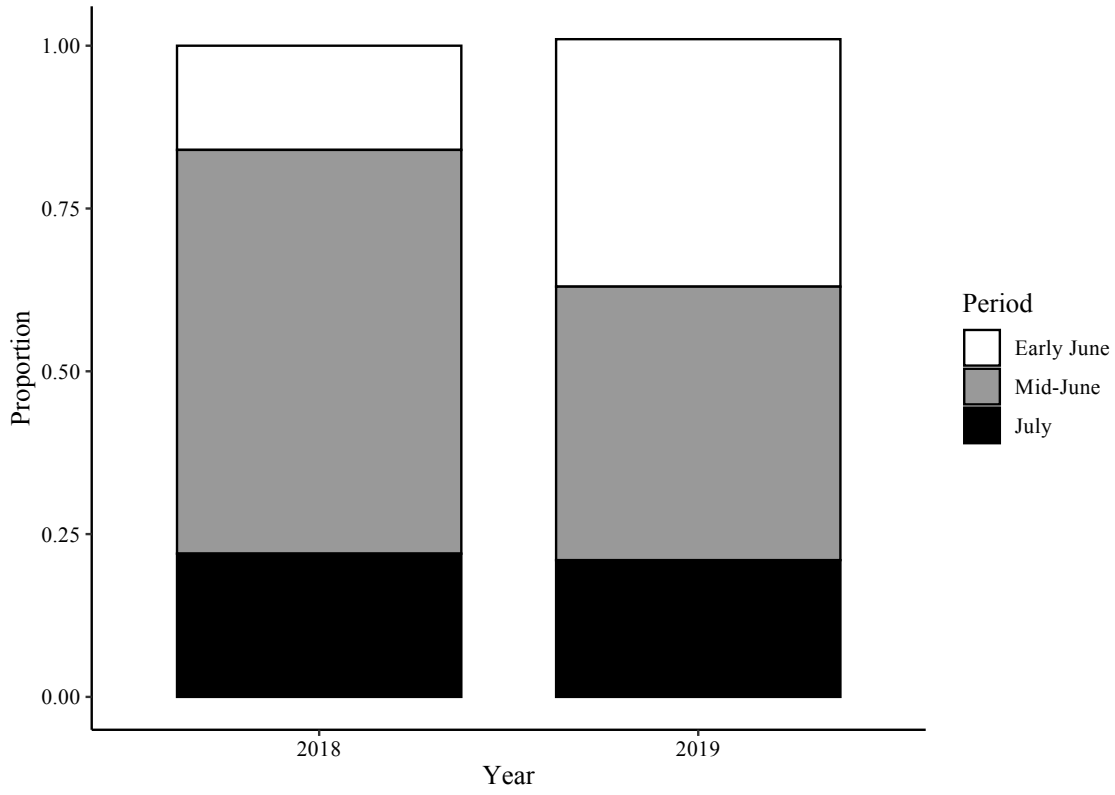

Figure 18. Within-year recapture occurrences at Veterans Memorial Wildlife Management Area.

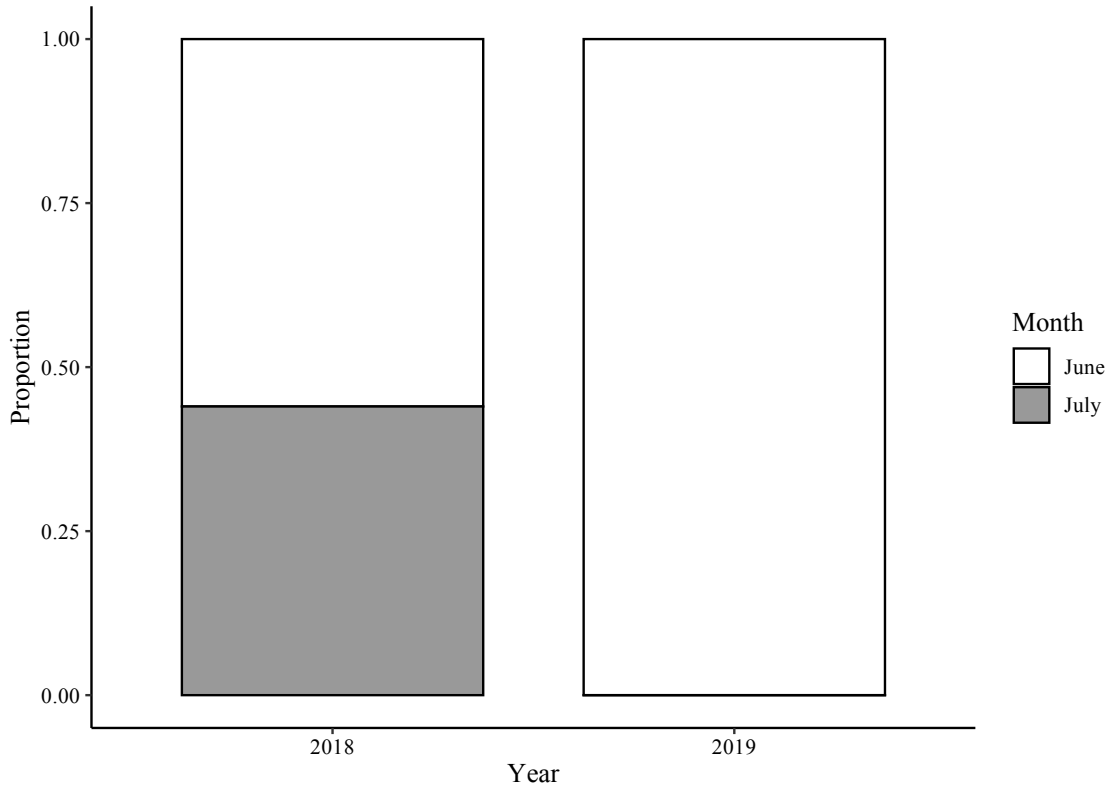

Figure 19. Within-year recapture occurrences at Yellowbank Wildlife Management Area.

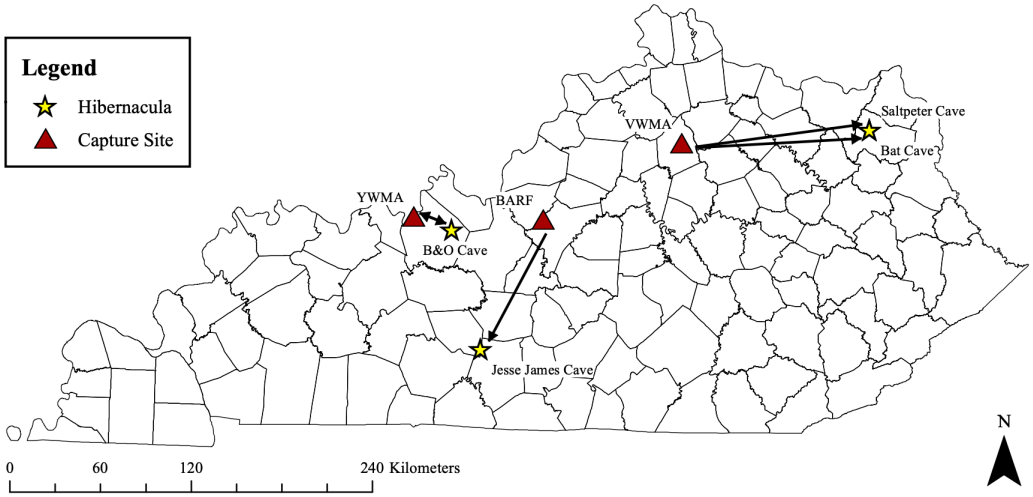

Figure 20. Kentucky hibernacula recaptures and general travel direction (depicted by arrows, exact travel route unknown) from capture location to overwintering site. Double-sided arrows represent instances of return captures.

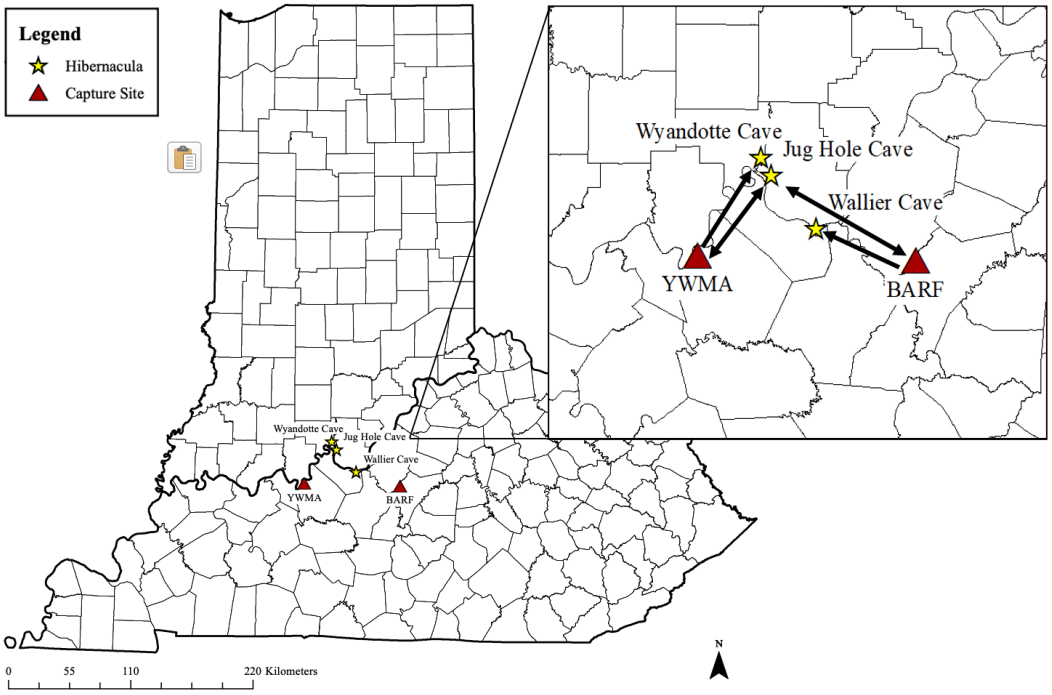

Figure 21. Indiana hibernacula recaptures and general travel direction (depicted by arrows, exact travel route unknown) from capture location to overwintering site. Double-sided arrows represent instances of return captures.

Figure 22. BrandenBark™ artificial roost structure at Veterans Memorial Wildlife Management Area with mesh guano catch installed below.

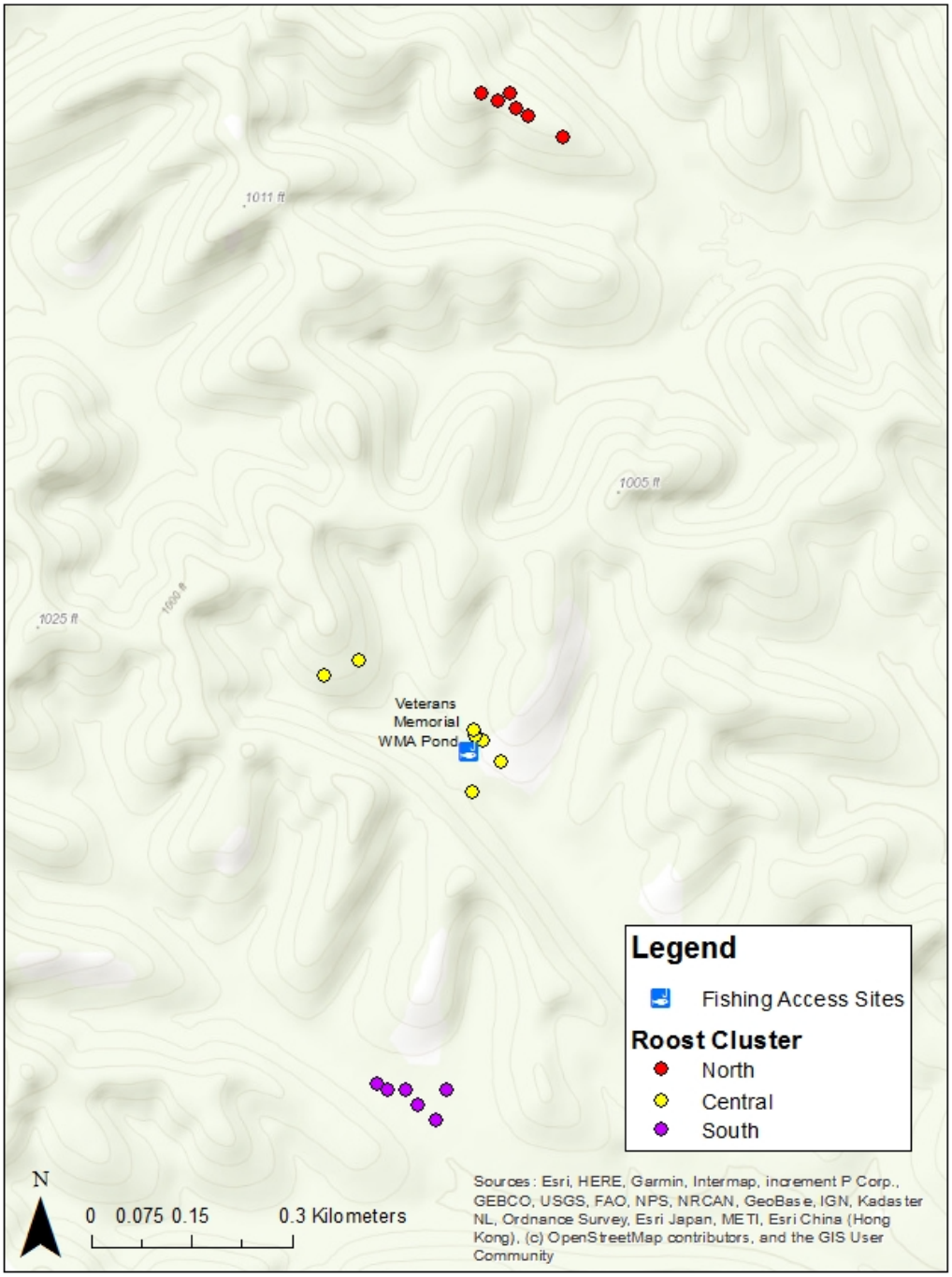

Figure 23. Roost clusters at Veterans Memorial Wildlife Management Area.

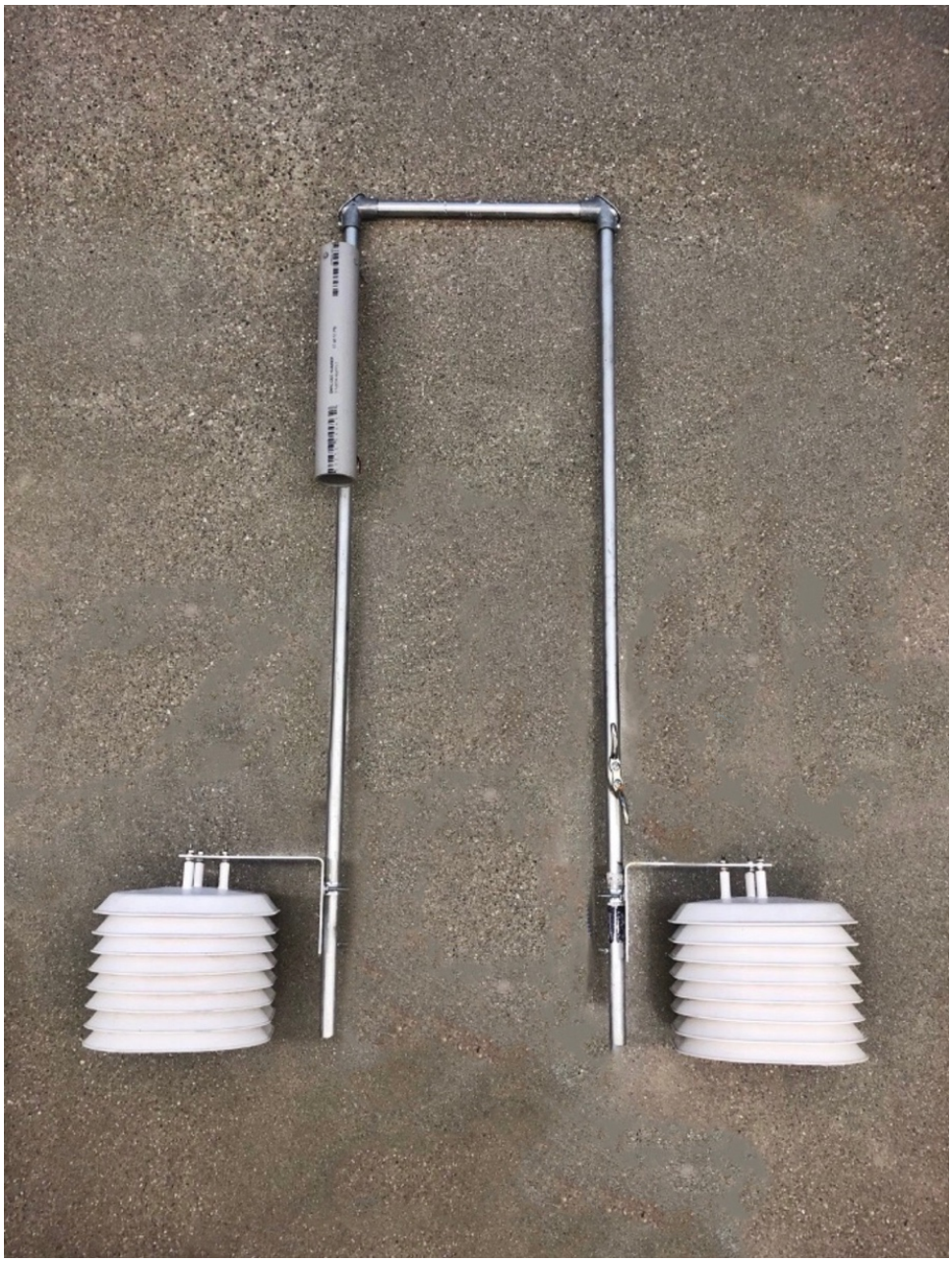

Figure 24. Data logger holster with attached solar radiation shields.

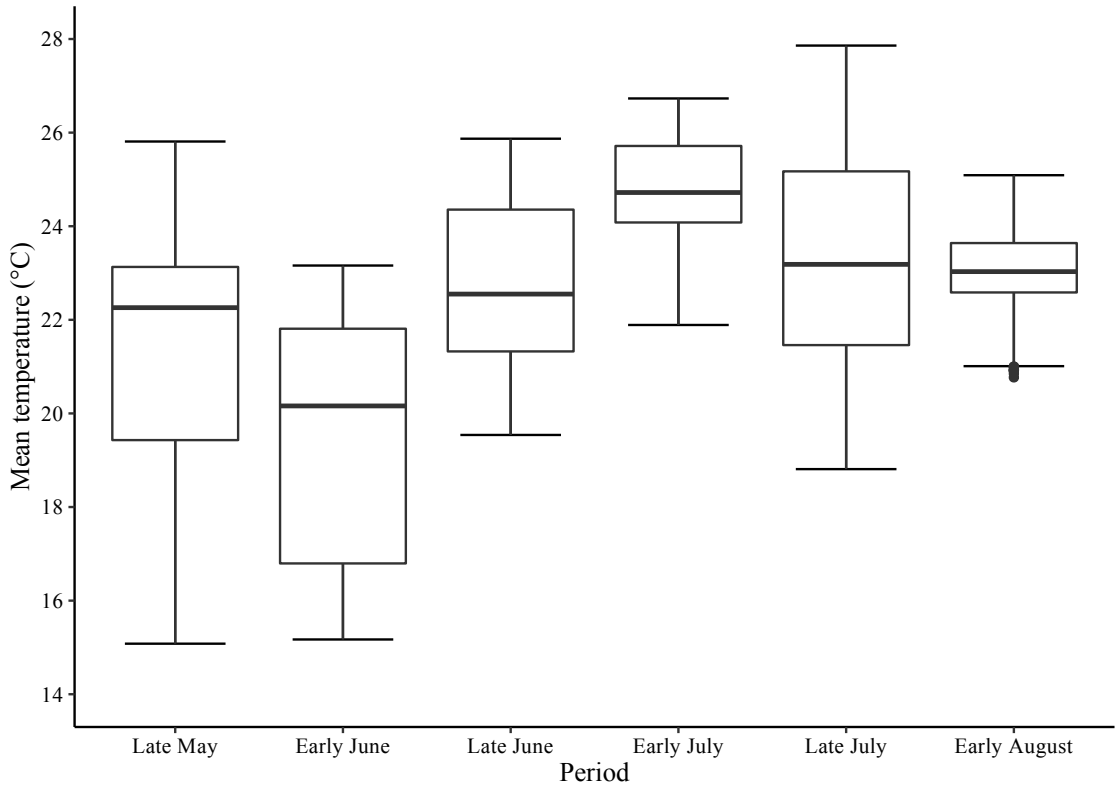

Figure 25. Boxplot of combined daily mean temperature for all roosts by two week periods through the summer maternity season.

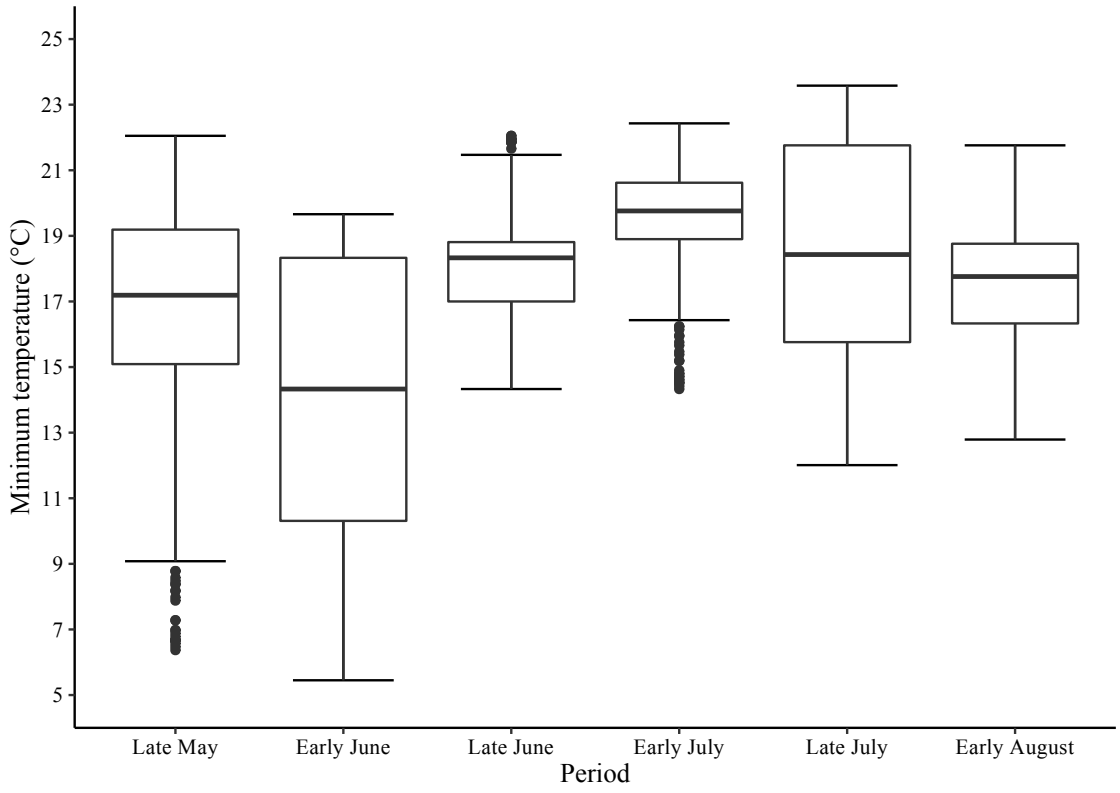

Figure 26. Boxplot of combined daily minimum temperature for all roosts by two week periods through the summer maternity season.

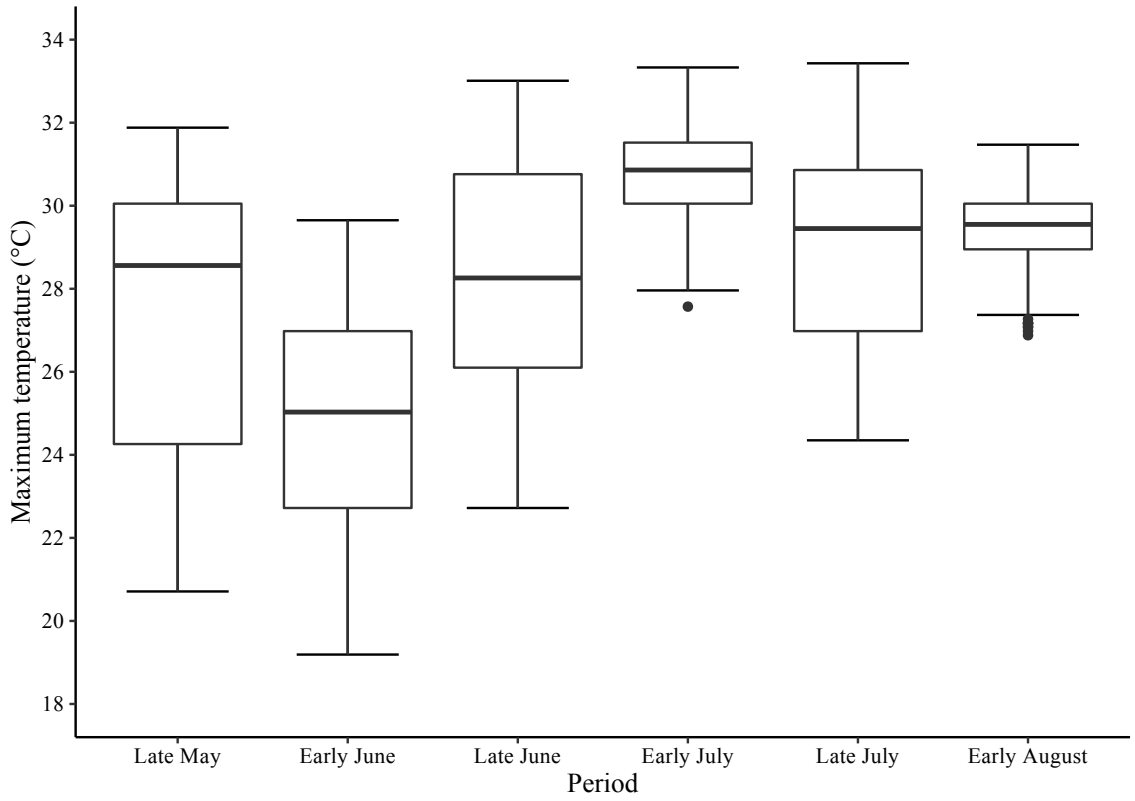

Figure 27. Boxplot of combined daily maximum temperature for all roosts by two week periods through the summer maternity season.

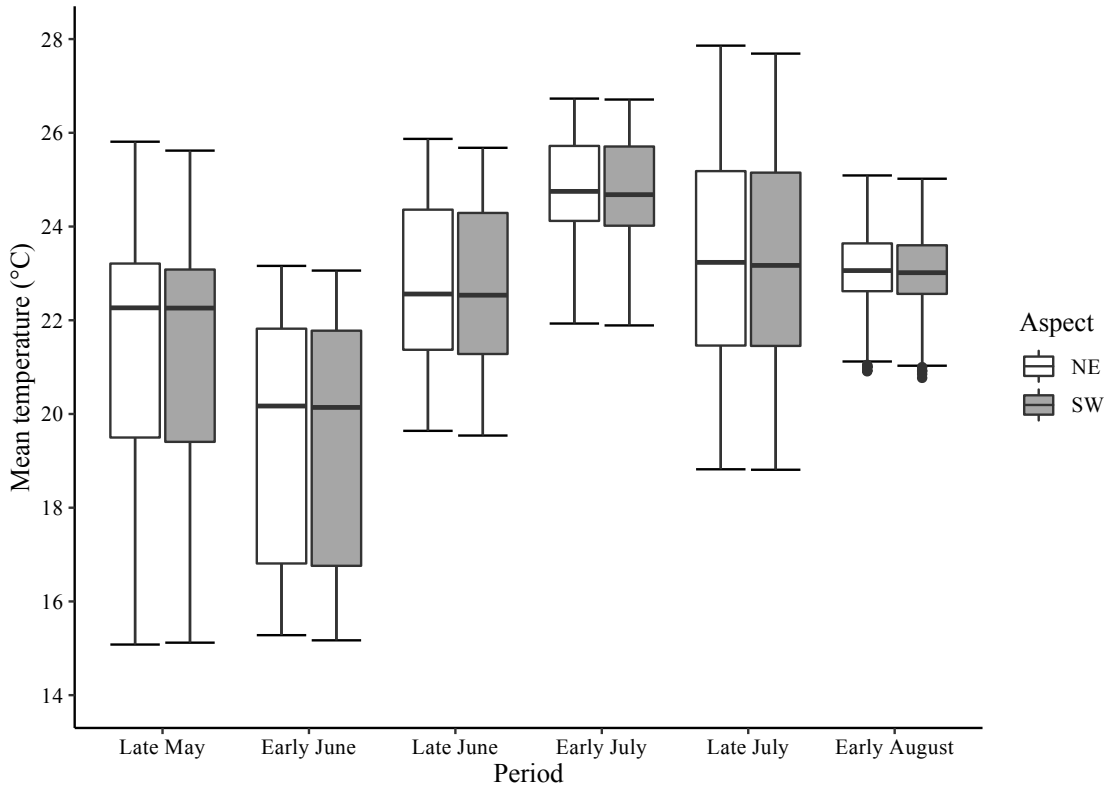

Figure 28. Boxplot of combined daily mean temperature by roost aspect across two week periods through the summer maternity season.

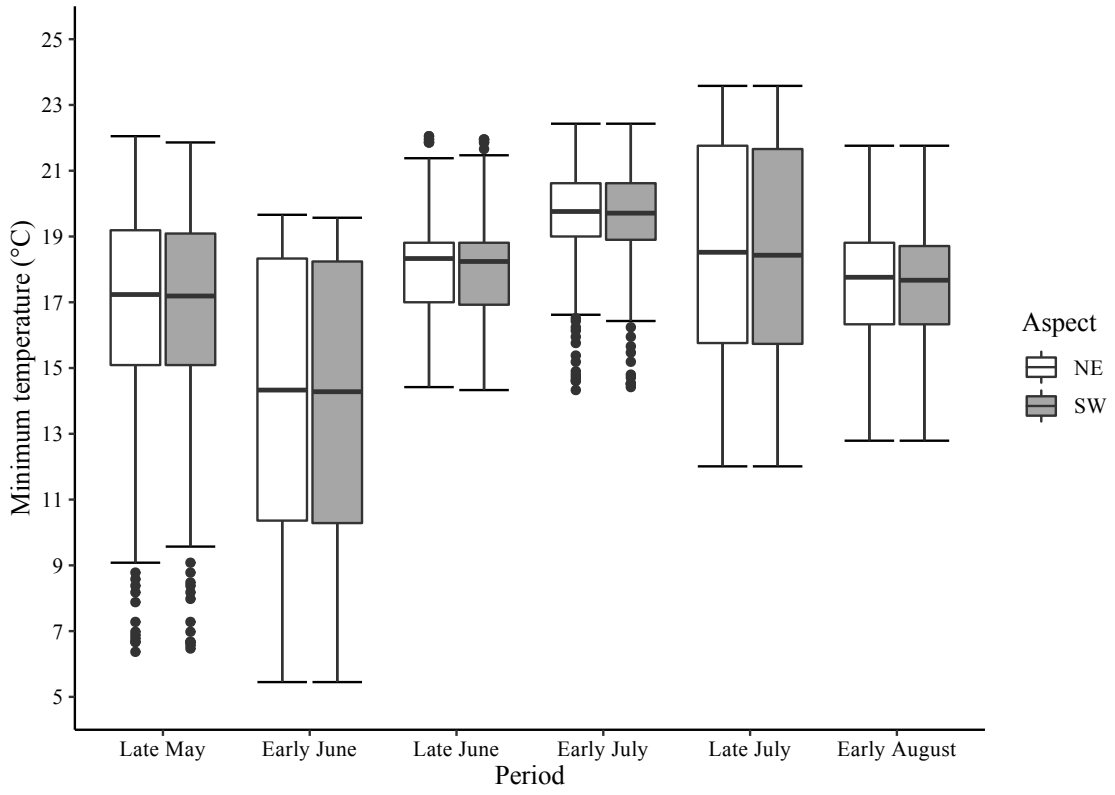

Figure 29. Boxplot of combined daily minimum temperature by roost aspect across two week periods through the summer maternity season.

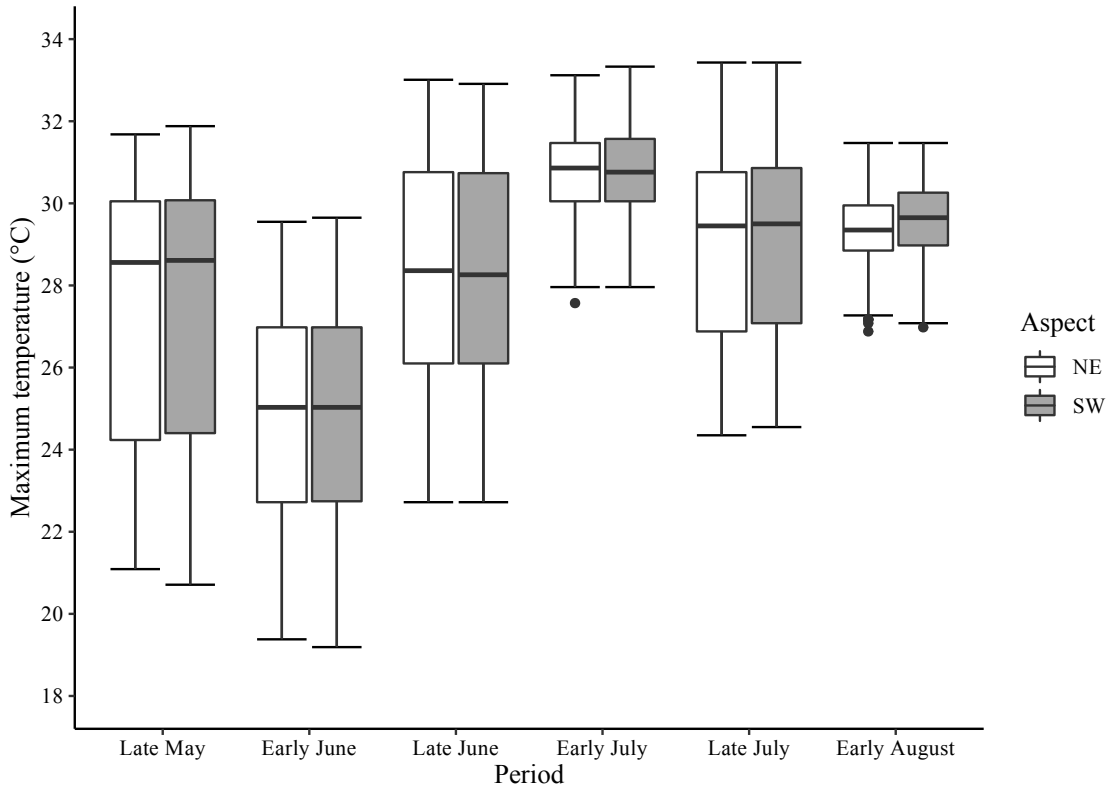

Figure 30. Boxplot of combined daily maximum temperature by roost aspect across two week periods through the summer maternity season.

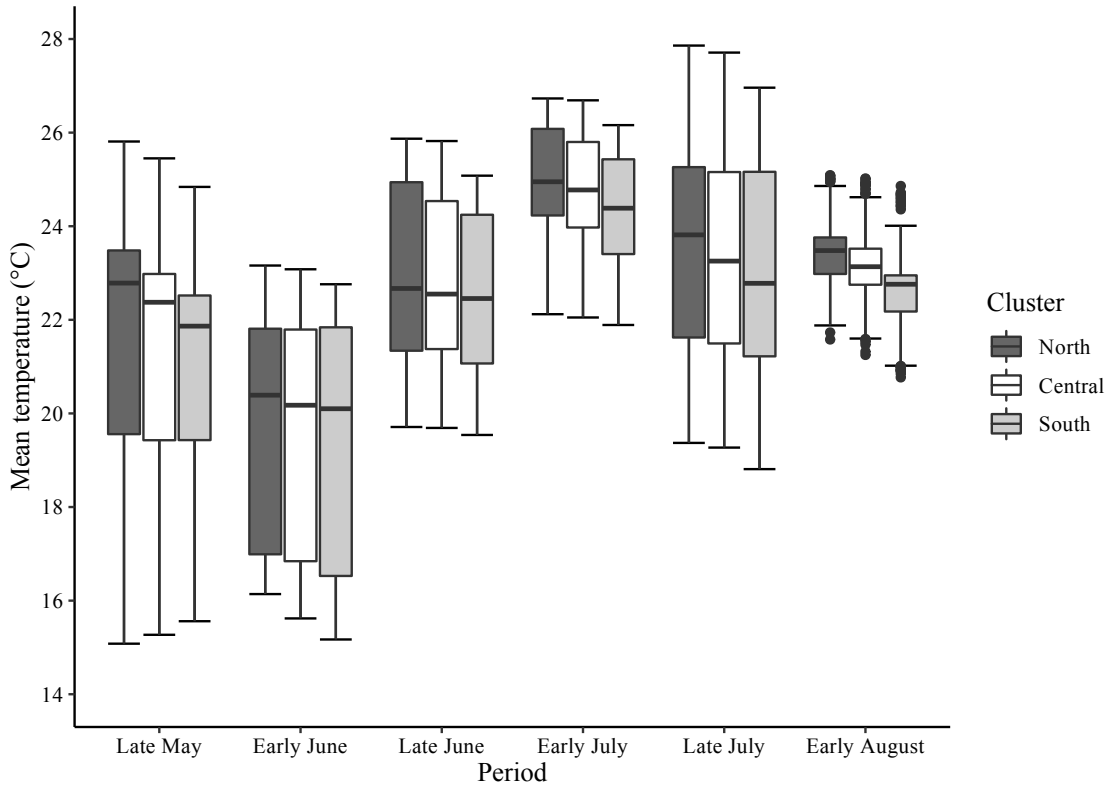

Figure 31. Boxplot of combined daily mean temperature of artificial roost clusters by two week periods through the summer maternity season.

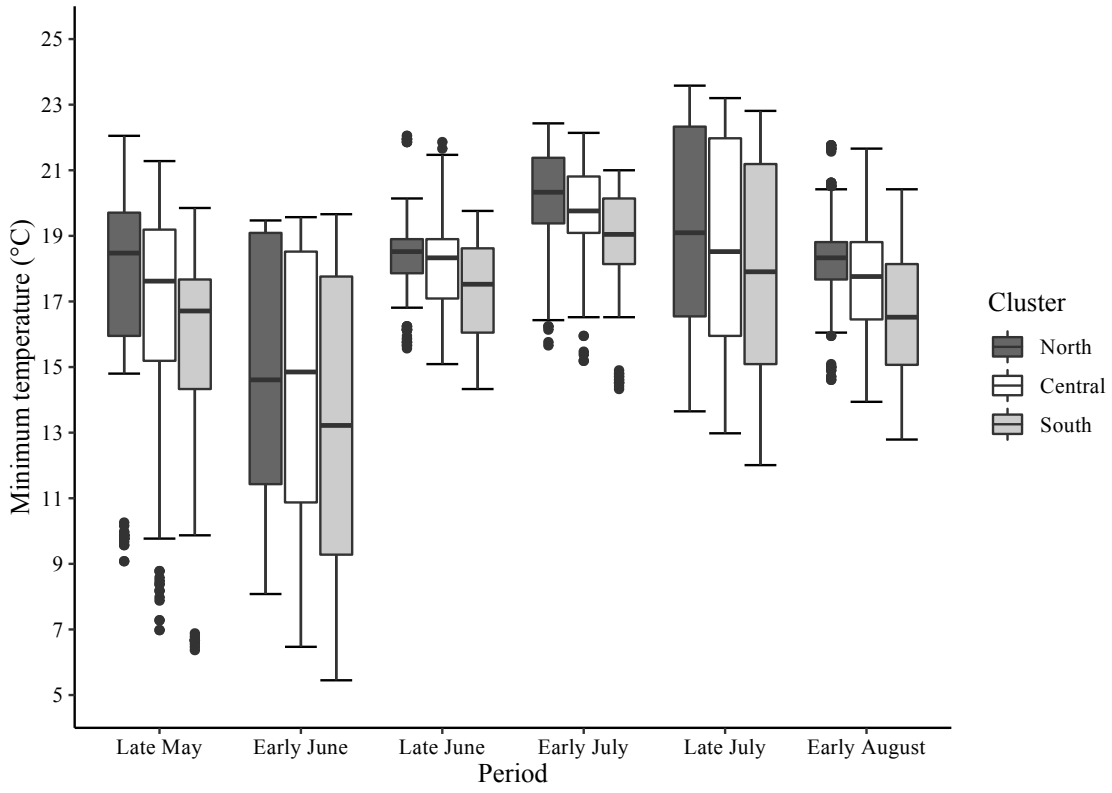

Figure 32. Boxplot of combined daily minimum temperature of artificial roost clusters by two week periods through the summer maternity season.

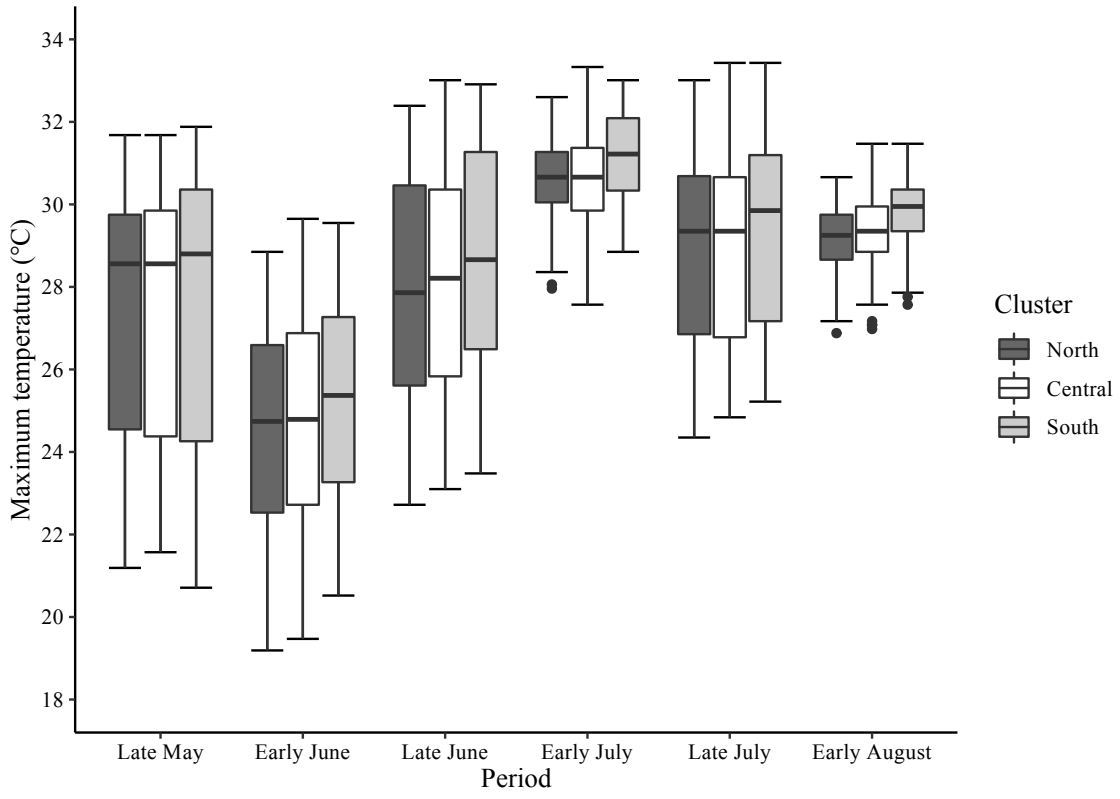

Figure 33. Boxplot of combined daily maximum temperature of artificial roost clusters by two week periods through the summer maternity season.

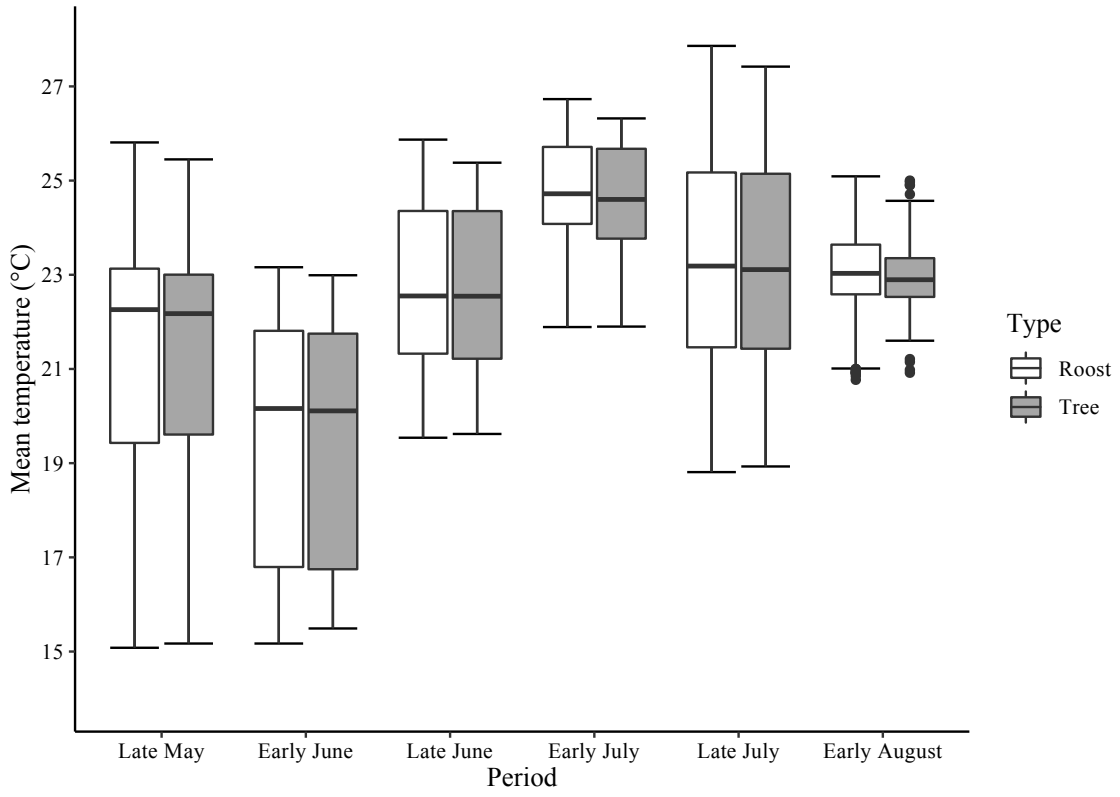

Figure 34. Boxplot of combined daily mean temperature of artificial roost and live tree loggers by two week periods through the summer maternity season.

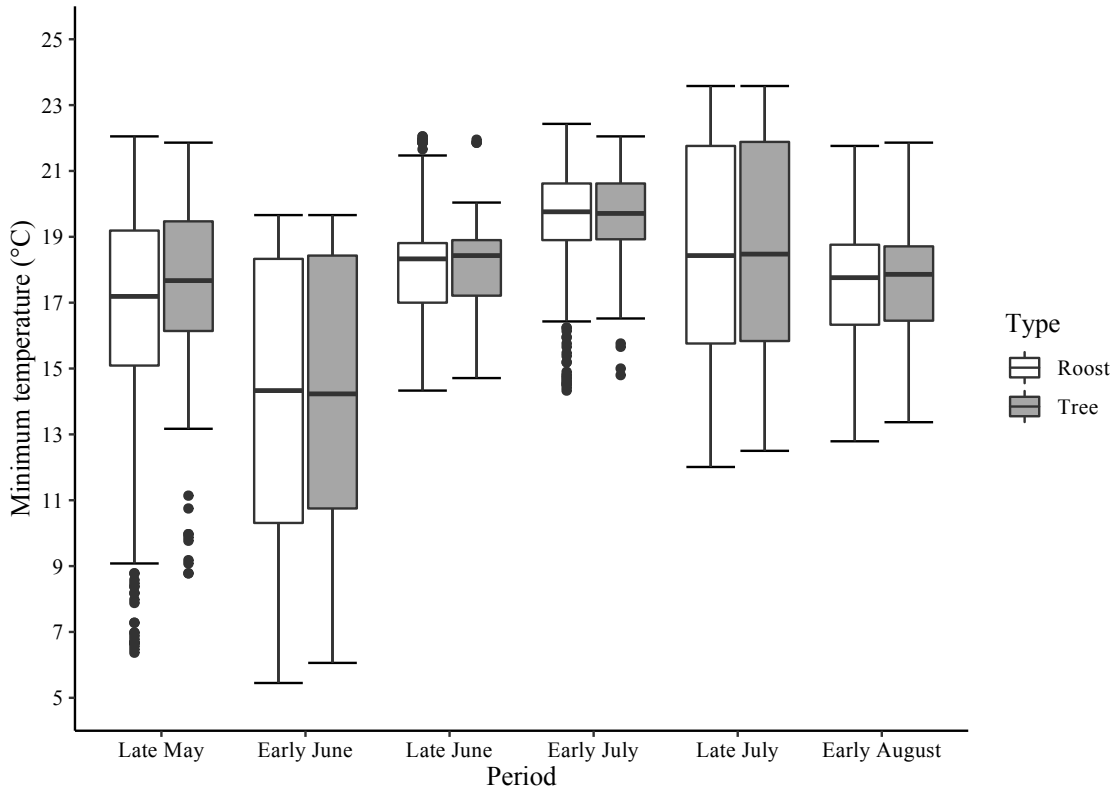

Figure 35. Boxplot of combined daily minimum temperature of artificial roost and live tree loggers by two week periods through the summer maternity season.

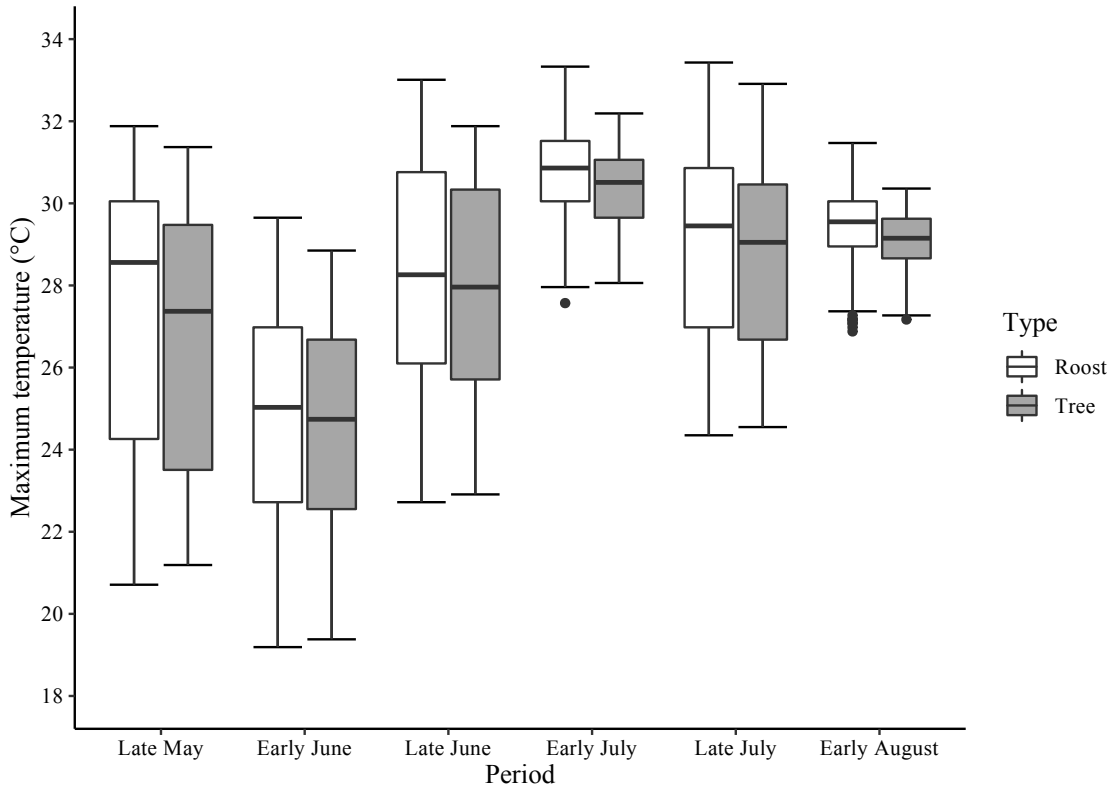

Figure 36. Boxplot of combined daily maximum temperature of artificial roost and live tree loggers by two week periods through the summer maternity season.

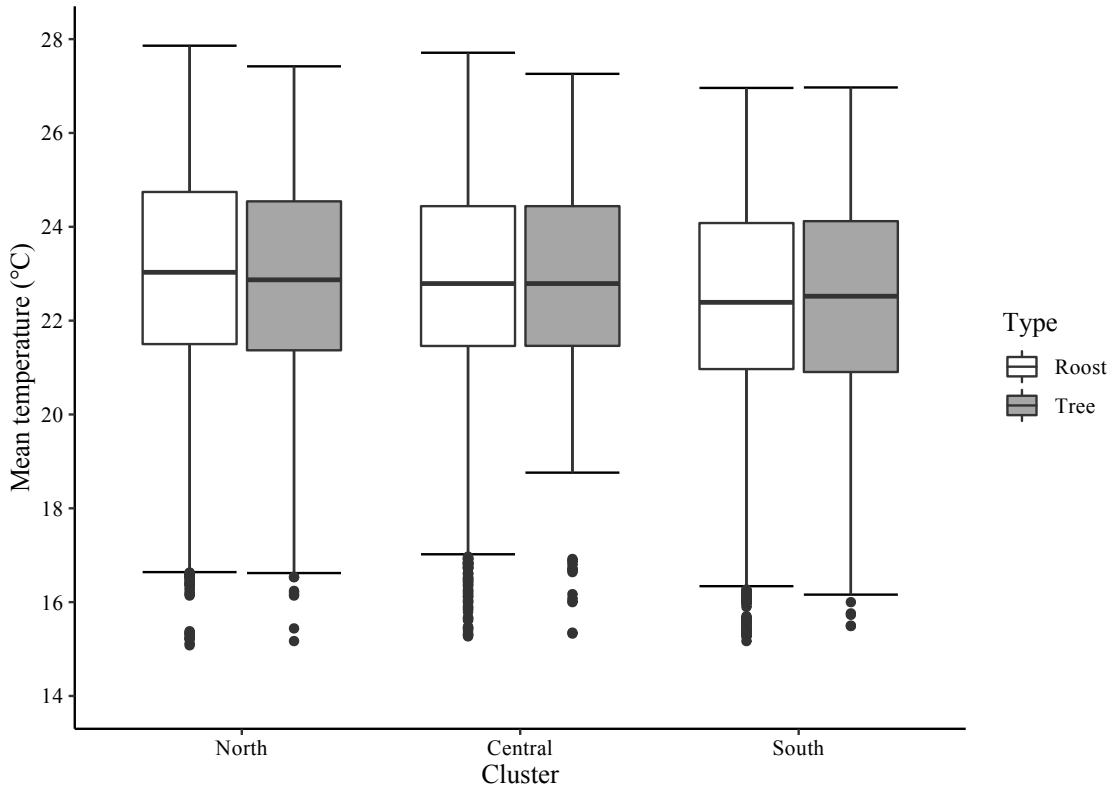

Figure 37. Boxplot of mean daily temperature of artificial roost trees and live trees by cluster.

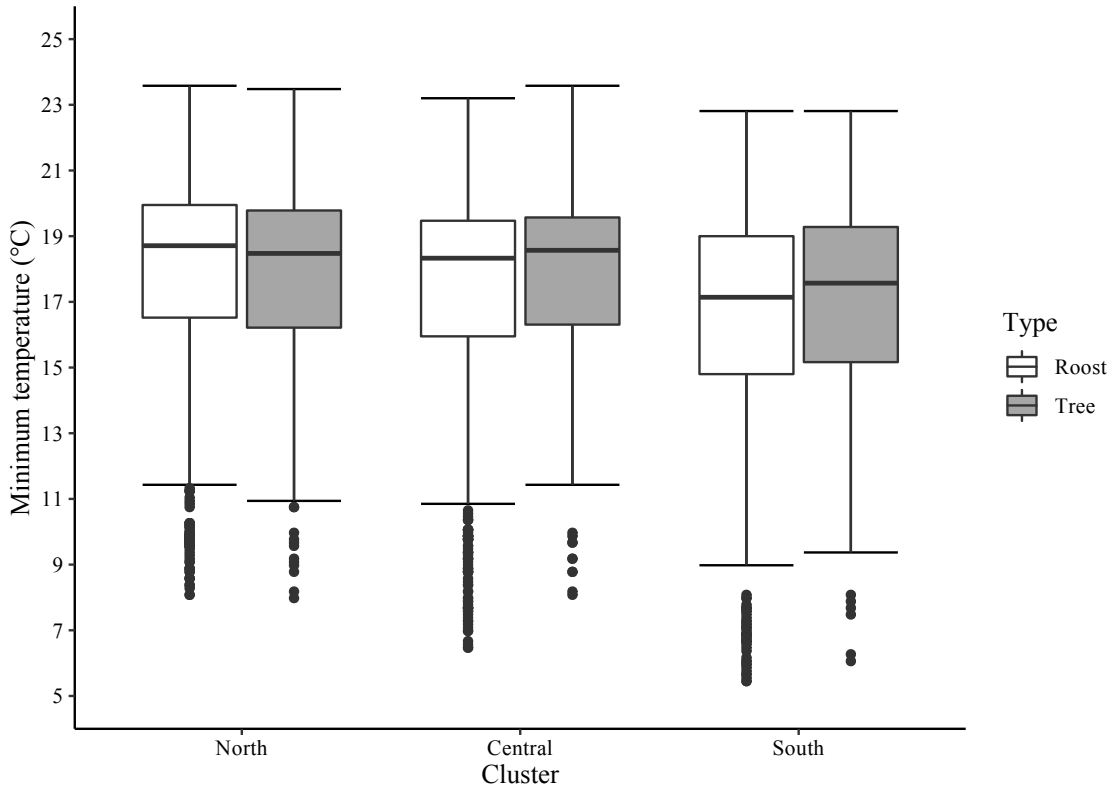

Figure 38. Boxplot of minimum daily temperature of artificial roost trees and live trees by cluster.

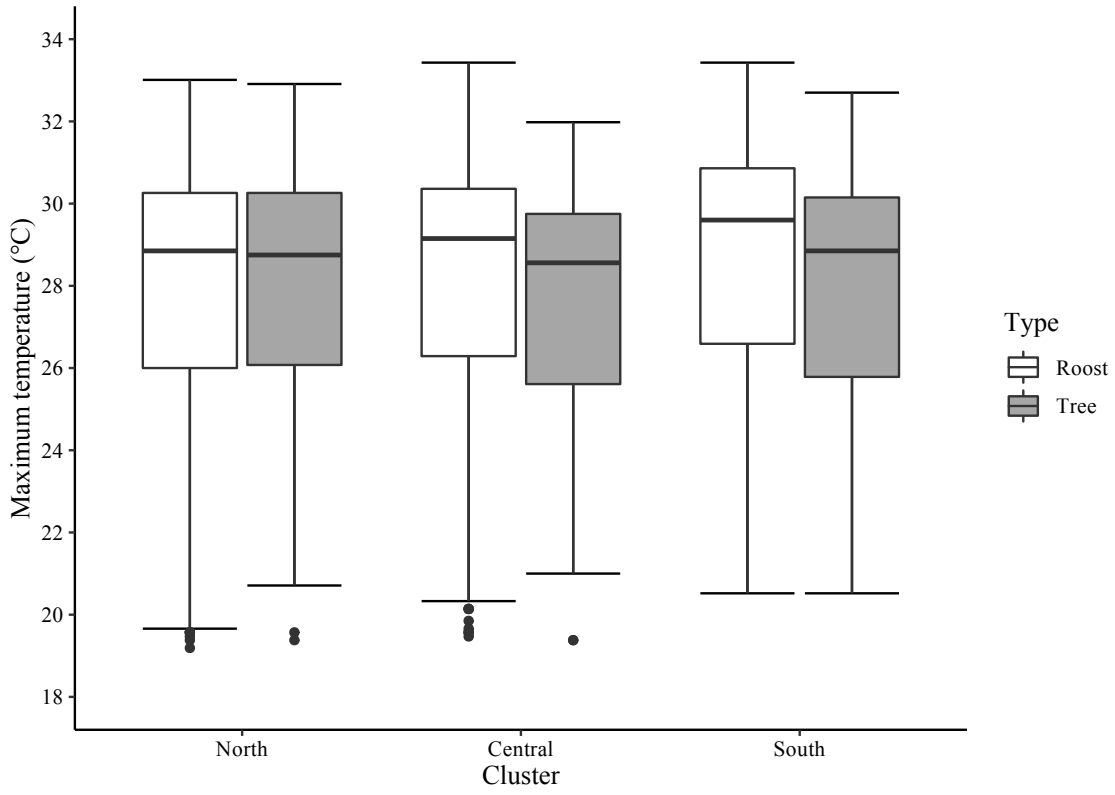

Figure 39. Boxplot of maximum daily temperature of artificial roost trees and live trees by cluster.

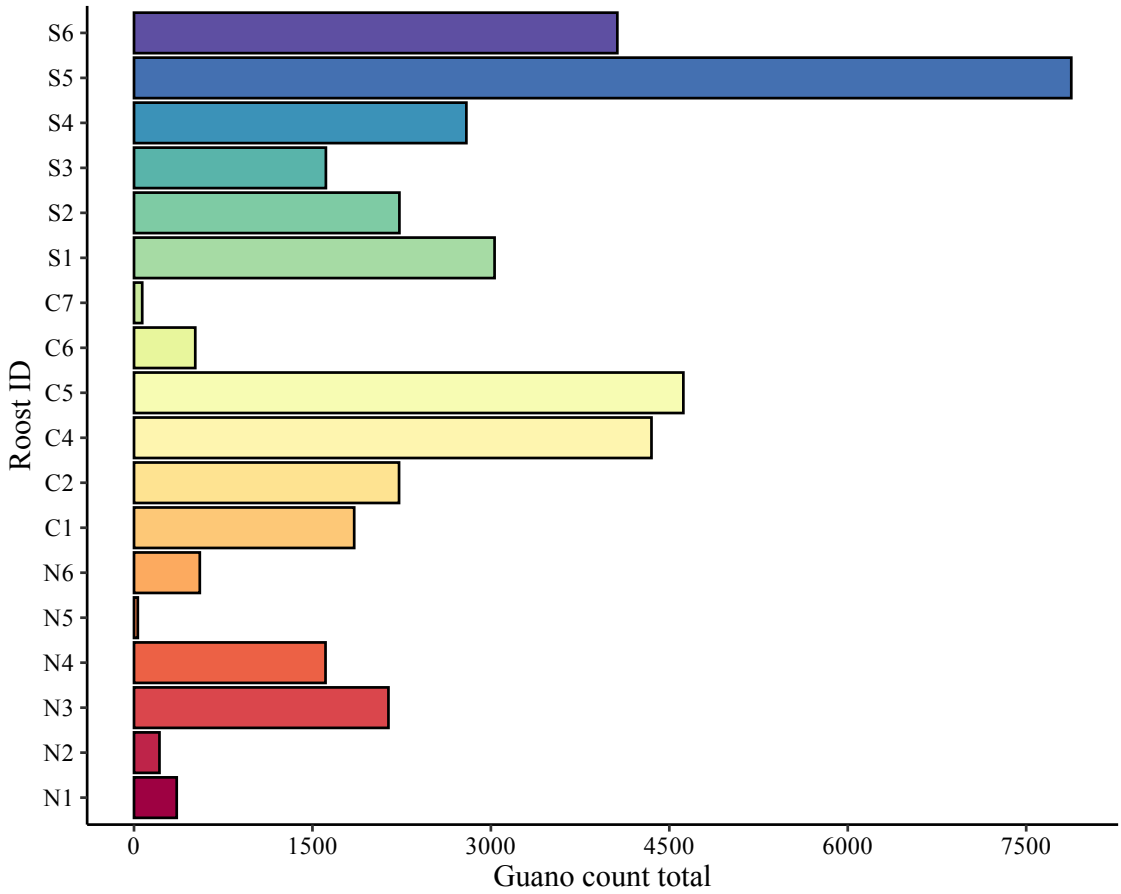

Figure 40. Total guano counts across individual roosts at Veterans Memorial Wildlife Management Area. Roost ID prefix letter is based on North, Central or South cluster.

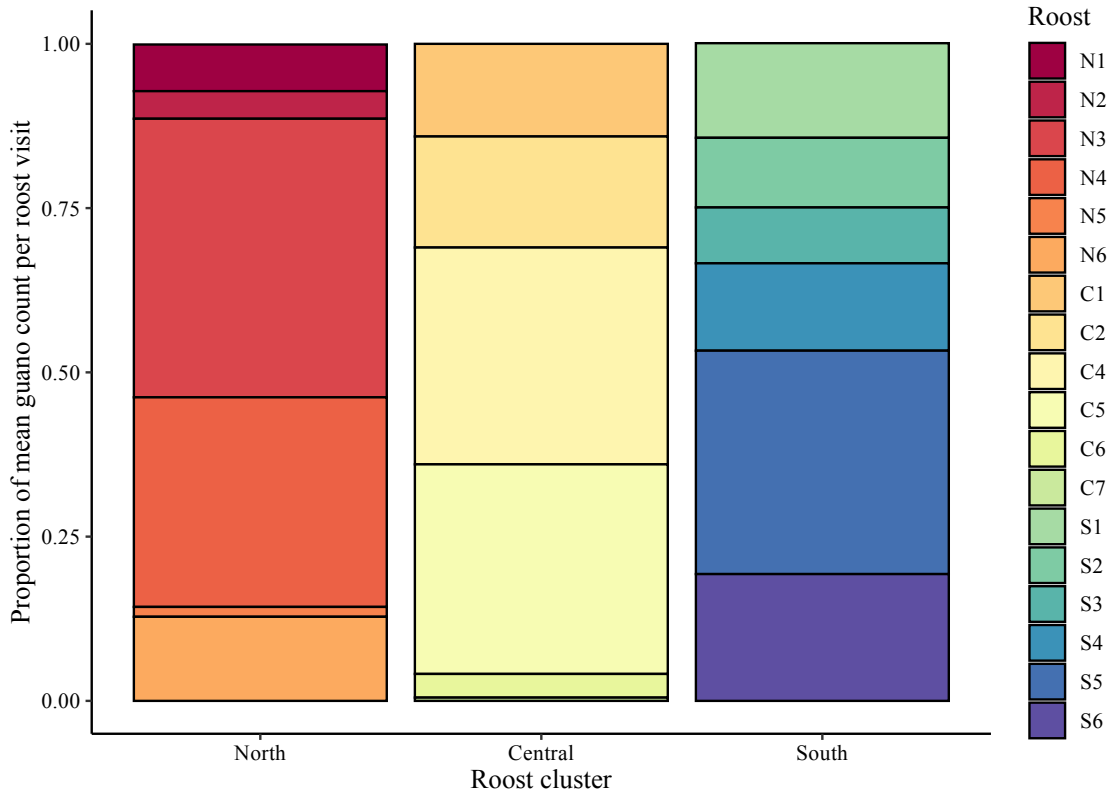

Figure 41. Proportion of mean guano count per roost within each of the three clusters at Veterans Memorial Wildlife Management Area.

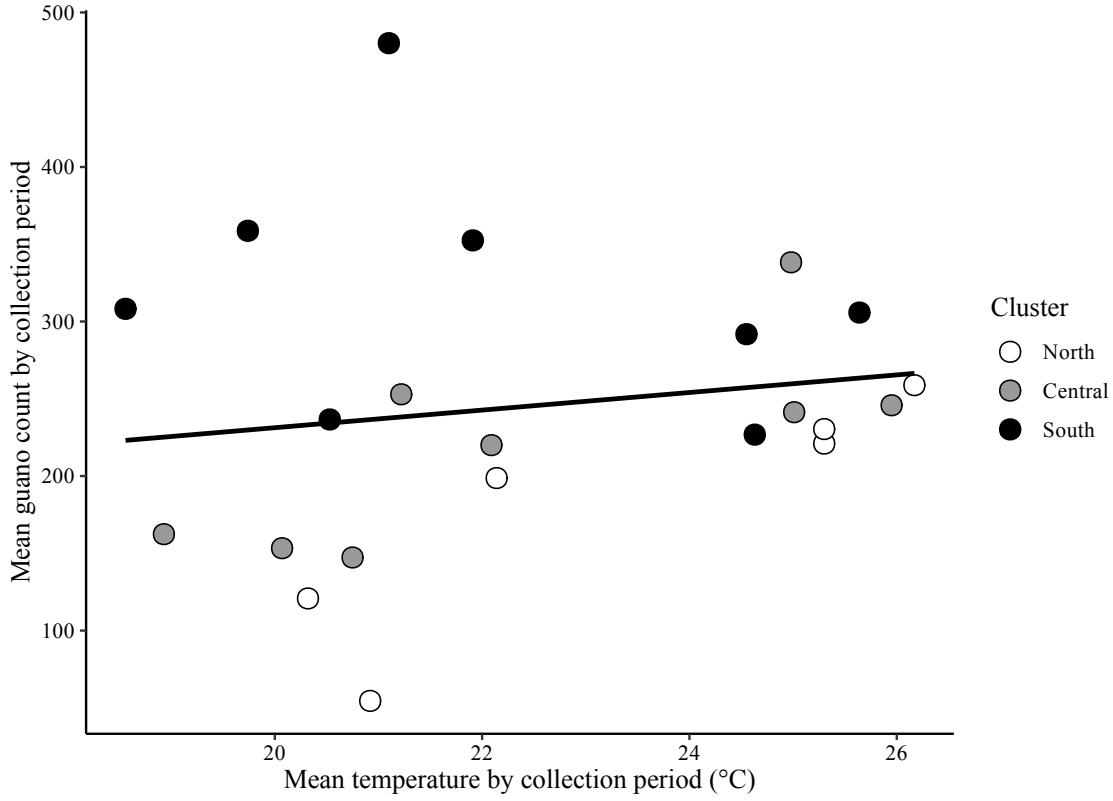

Figure 42. Mean collection period guano count by mean temperature compared by cluster.

Period	Julian date
1	149-151
2	160-163
3	165-168
4	170-172
5	174-176
6	178-179
7	180-182
8	186-188

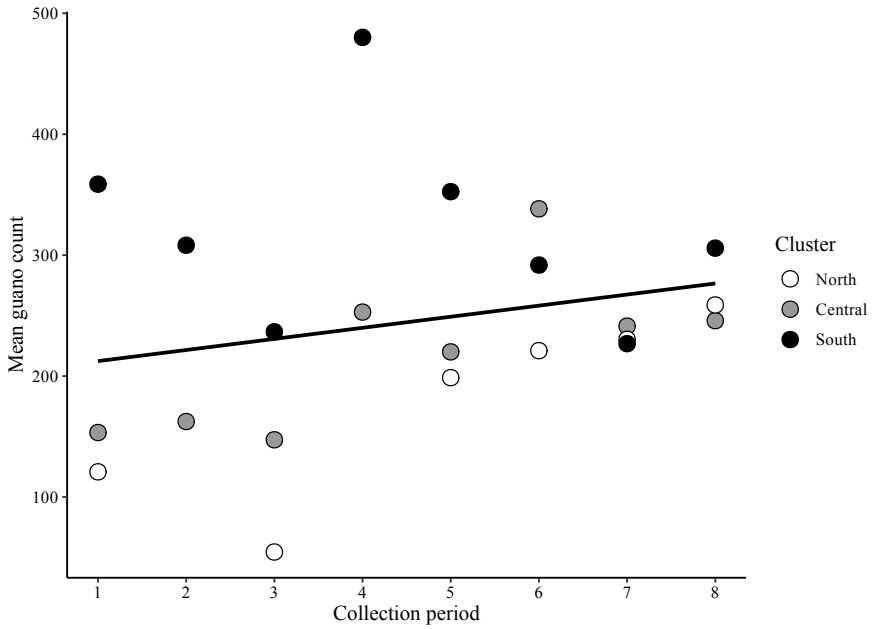

Figure 43. Mean guano count by cluster across guano collection periods (Julian date).